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Systematics, Phylogeny and Biology of the Neogastropoda

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(Marco Oliverio & Renato Chemello, eds)

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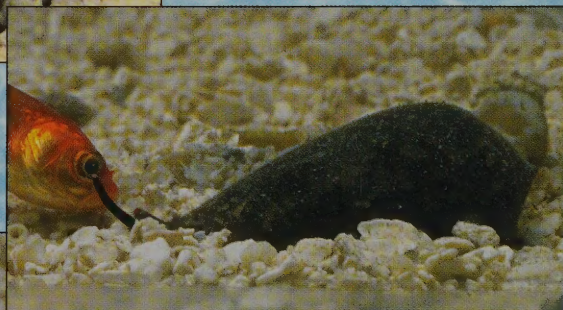
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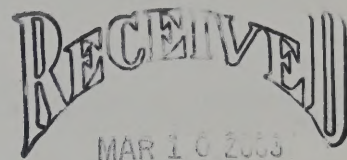
Systematics, Phylogeny and Biology of the Neogastropoda

Marco Oliverio & Renato Chemello (eds)

FOREWARD

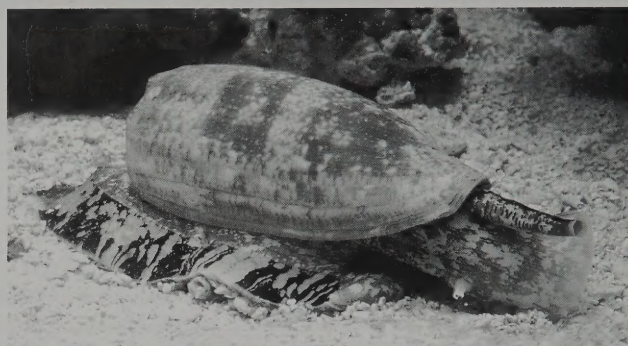
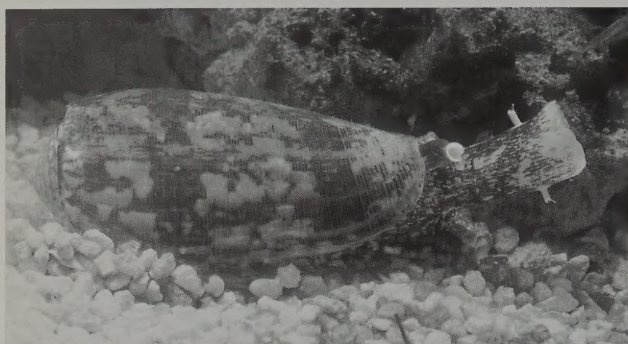
Proceedings to the *3rd International Workshop of Malacology*, held in Menfi (14-18 June 2000) Istituzione Culturale Federico II

The idea of gathering a number of persons dealing with the Neogastropoda under several distinct (though interconnected) perspectives, arose during the final meeting of the 2nd International Workshop on Malacology, centred on the opisthobranchs (Cervera & Cattaneo Vietti, 2000). The observation that a significant increase of knowledge had been accumulated during the last years on this group by several teams around the world (e.g.: BALL *et al.*, 1997, 1997a; HARASEWYCH *et al.*, 1997; KANTOR & TAYLOR, 1991; TAYLOR, KANTOR & SYSOEV, 1993), suggested to take the occasion to trace the state of the art and to draw the possible future development.
Six invited lectures, and seventeen communications/posters covered three main fields, providing high level reviews and original scientific results on three main topics:
1. The phylogenetic hypotheses on the origin and radiation of the Neogastropoda.
2. The biology of the neogastropods.
3. The systematics of the several subgroups in the Neogastropoda.

Thirteen papers have been finally accepted for the publication in the present proceedings volume.

1. The phylogenetic hypotheses on the origin and radiation of the Neogastropoda with data from morphology, DNA, paleontology, development and ecology

Yuri I. Kantor summarised the existing hypotheses on neogastropod radiation and showed the possible sequence of morphological transformations of their digestive system. He suggests that the differing opinions on the neogastropod evolution (e.g.: KANTOR, 1996; AMAUDRUT, 1898; PONDER, 1974; GOLIKOV & STAROBOGATOV, 1988; PONDER & LINDBERG, 1997; RIEDEL, 2000)) are likely the result of high rate of homoplasy within the neogastropods, since a large number of lineages rapidly proliferated during the Cretaceous (HARASEWYCH *et al.*, 1997). The result is a lot of differences in the opinions on the position of most families, as well as in the treatment of characters and character states. With relatively few morphological novelties recognizable, a cladistic analysis of the neogastropod lineages is often unsatisfactory, or poorly resolved (eg. KANTOR, 1996). The lack of clear autapomorphies hampers in some cases to recognize as monophyletic taxa, groups of species characterised by combination of non-exclusive characters (as in the case of the : SMITH, 1998 vs. KANTOR, 1991). In these cases the availability of a molecular dataset often helps, providing a molecular framework within which the evolution of the morphological characters can be analysed. The rapid adaptive radiation of the neogastropods is probably best affordable by the sequences of genes like the cytochrome *c* oxidase I gene (HARASEWYCH *et al.*, 1997). The analysis of the evolution of the digestive system in the neogastropods supports their monophyly by several synapomorphies in the anterior foregut. Also the ontogenetic data give support to the neogastropod monophyly, as shown by Alex Ball with the similar developmental pattern of the foregut in *Conus anemone* and *Nucella lapillus*. Heterochrony associated to a considerable plasticity can give reason for the major differences in neogastropod adult morphology. The definition of the plesiomorphic state for some characters - the most primitive neogastropods have short proboscis, basal buccal mass, and the odontophoral and radular muscles pass through the nerve ring and join the columellar muscle - provide good indication for determining the potential sister groups of the Neogastropoda. According to Yuri I. Kantor, it should be searched for among the carnivorous groups of the Sorbeoconcha with underived foregut.

2. The biology of neogastropods, with special emphasis on the evolution of the conotoxins and their use in pharmacology

The five communications dealing with the biology of the neogastropods span a good deal of the taxonomic diversity: muricoideans, buccinoideans and conoideans are all treated.
The biology of cones is explored by Alan Kohn in the light of phylogeny to indicate how interpretation of the results differs in the absence of phylogenetic information. The group originated probably around the Lower Eocene and radiated first in the Middle Eocene. In the Cainozoic times of rapid radiation were punctuated by periods of reduced diversity, in a fashion similar to that of other invertebrates, addressing to extrinsic evolutionary factors. Kohn shows how ecological data may have a strong phylogenetic signal, with species within clades identified by molecular studies, using similar substrate and prey types. An analysis of the evolutionary pathway for the radula of cones has been presented by Rolán & Raybaudi, who propose the identification of the plesiomorphic vs. apomorphic state in radular character states. The mechanism of envenomation by cones has been explained by Detomal Espiritu *et al.* who have reviewed the present knowledge and the expanding application of cone snail venom components in medicine. They have furthermore addressed a very promising issue: the degree to

which the envenomation strategy may be shared by other venomous gastropod groups.
Helena Fortunato uses a comparative dataset on the reproduction and larval development of the *Strombina*-group and related gastropods to test the use of the larval shell for inference of development in fossil species. Having such comparative fossil-Recent studies available for more groups would provide sounding basis for the use of biometric data of the larval shells in fossils to infer developmental strategies.
Three further communications show how little we know about the biology of the neogastropods. Tan & Oh reported on the feeding habits of *Chicoreus capucinus*, a common predator in Indo-Pacific mangroves. Despite its large size and common occurrence, its biology and feeding habits are poorly known, when compared to rapanine counterparts. Solustri *et al.* described the biometrics of *Nassarius mutabilis*, a very common and commercially important Mediterranean gastropod. Hergueta *et al.* reported on the taxonomy, ecology and biology of *Chauvetia mamillata*, that is reported as feeding on egg capsules of other gastropods.

3. The systematics of several subgroups in the Neogastropoda with data from morphology, DNA, paleontology, development and ecology

M.G. Harasewych & Yuri I. Kantor described for the first time in detail the external morphology and anatomy of several species of the commercially important genus *Babylonia.* Among other characters, the radula of all species differs markedly from that of any buccinoidean. Anatomy and DNA (partial sequences of CO-I) showed that *Babylonia* has close affinities to Volutidae and Olividae. The family Babyloniidae Kuroda, Habe and Oyama, 1971 is thus restored.
Guido Pastorino revised the systematics and phylogeny of the genus *Trophon* from Patagonia and Antarctica, based on the examination of over 1,000 specimens, in more than 600 lots. Four questions are addressed: how many valid *Trophon* species live in Patagonia and Antarctica? What is their range? How many lineages are represented? Do they form a monophyletic unit? It is suggested that the Patagonian species group and the Antarctic species group heretofore considered to be in the same genus, are probably polyphyletic.
Alexandra Richter & Ángel A. Luque provided a very up-to-date compilation of the current knowledge about feeding, anatomy, sexual strategy, parental care and protoconch of the coralliophilid gastropod. They show the results of a preliminary cladistic analysis on 25 characters relative to the anatomy, reproductive biology and larval development. This analysis separates Coralliophilidae and Muricidae into two independent monophyletic clades, and divide the coralliophilids into a primitive clade and a more derived one, a result that contrasts recent molecular analyses (Oliverio & Mariottini, 2001). The need for a deepening in the study of the anatomy and biology of coralliophilids is stressed, in particular in the reproductive system and reproductive strategy.

Raphitominae are morphologically the most diverse subfamily of conoideans. The great variation in the foregut anatomy results in three main feeding modalities: 1) the normal toxoglossan feeding with the radular tooth at the tip of the proboscis used to sting and inject the venom; 2) the use of the venom apparatus without the use of the radula; 3) prey capture without radula and venom, possibly by suction. A phylogenetic analysis suggests that Raphitominae have close affinities with Coninae and Mangeliinae.

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We wish to take this occasion to express all our gratitude to the "Istituzione Culturale Federico II" of Menfi, that hosted and supported logistically the workshop. The city of Menfi, the Regional Province of Agrigento supported the organization and the publication of this volume. The Cantine Settesoli sponsored the workshop. The series of International Workshops of Malacology would not be possible if it was not for the enthusiastic work of Ms. Vanna Rotolo: we are all indebted with her, and wish to dedicate this volume to her continuous and generous effort to have malacology promoted in Sicily.

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Current knowledge on Coralliophilidae (Gastropoda) and phylogenetic implication of anatomical and reproductive characters

Alexandra Richter & Ángel A. Luque

KEY WORDS: Gastropoda, Coralliophilidae, feeding, anatomy, sexual strategy, parental care, protoconch, phylogenetic relationships.

ABSTRACT The current knowledge about feeding, anatomy, sexual strategy, parental care and protoconch of Coralliophilidae (Gastropoda) is reviewed. A preliminary cladistic analysis is carried out in order to unravel the phylogenetic relationship within Coralliophilidae and among Coralliophilidae and Muricidae. Twenty five characters and 55 character states chiefly relative to the anatomy, reproductive biology and larval development has been used in the analysis. The main result of the analysis is the separation of Coralliophilidae and Muricidae into two independent monophyletic clades, and the division of Coralliophilidae into a primitive clade represented by *Coralliophila squamosa*, with no known synapomorphies, and a more derived one, which give rise to three evolutionary lines represented by the genera *Babelomurex, Coralliophila* and *Leptoconchus*. This latter clade is characterised by the synapomorphy of brood care and by further four potential synapomorphies pertaining to the anatomy of the reproductive system. The internal relationships of Muricidae revealed by the present analysis support other current phylogenetic hypotheses obtained by analysis using anatomical or molecular characters. In conclusion, the paper stresses the necessity in deepening in the study of the anatomy and biology of coralliophilids, in particular in the reproductive system and reproductive strategy, since these aspects have been shown to be important in establishing internal relationships of coralliophilids.

RIASSUNTO Viene riportata una revisione delle conoscenze attualmente disponibili su strategie alimentari e sessuali, anatomia, cure parentali e protoconche delle Coralliophilidae. Un'analisi cladistica preliminare è condotta col fine di dipanare le relazioni filogenetiche all'interno del gruppo e tra le Coralliophilidae e Muricidae. Sono stati usati nell'analisi venticinque caratteri per 55 stati, principalmente relativi all'anatomia, alla biologia riproduttiva e allo sviluppo larvale. Il risultato principale dell'analisi è la separazione di Coralliophilidae e Muricidae in due linee monofiletiche indipendenti, e la divisione delle Coralliophilidae in una linea primitiva rappresentata da *Coralliophila squamosa*, ed un clado più evoluto che ha dato origine a tre linee rappresentate dai generi *Babelomurex, Coralliophila* e *Leptoconchus*. Quest'ultimo clado è caratterizzato dalla sinapomorfia dell'incubazione delle capsule ovigere e da ulteriori quattro potenziali sinapomorfie riguardanti l'anatomia dell'apparato riproduttore. Le relazioni interne ai Muricidae rivelate da quest'analisi supportano altre ipotesi filogenetiche correnti derivate da dati anatomici e molecolari. In conclusione, il lavoro evidenzia la necessità di un approfondimento nello studio dell'anatomia e della biologia dei coralliofilidi, in particolare per ciò che riguarda l'apparato riproduttore e le strategie riproduttive, in quanto questi aspetti hanno dimostrato la loro importanza nello stabilire le relazioni interne tra i coralliofilidi.

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INTRODUCTION

Coralliophilids are marine neogastropods that live in tropical to temperate waters and feed exclusively on anthozoans. Up to date about 200 recent species have been recognised and grouped into 10 genera (Kosuge & Suzuki, 1985; Vaught, 1989) according to shell and opercular characters. However, since the shell of coralliophilids shows a great interspecific and intraspecific heterogeneity, the current generic and specific classification has to be considered as provisional and awaits for a critical review that should consider anatomical, reproductive, developmental and ecological aspects, on which scarce data exist. The lack of information on these aspects also has made it difficult to unravel the relationship of coralliophilids with other neogastropod families. Traditionally, Coralliophilidae has been considered a group closely related to Muricidae (Thiele, 1929; Ponder, 1973) due to their similarity in shell characters and external anatomy. However, in a first attempt to assess the phylogenetic relationships of neogastropod families by means of a cladistic analysis based on characters of the alimentary tract, Kantor (1996) failed to demonstrate such affinity. This assumed close relationship between Muricidae and Coralliophilidae has been supported recently by the results of phylogenetic analysis using DNA sequences coding for cytochrome c oxidase I (Harasewych *et al.,* 1997) and 12 S rDNA sequences (Oliverio & Mariottini, 2001a). Oliverio & Mariottini (2001a) even proposed to rank coralliophilids as a subfamily within Muricidae on the basis of the outcome of their phylogenetic analysis that revealed the coralliophilids as an evolutionary line within the Muricidae with Rapaninae being their sister taxon.

Since there are marked differences among coralliophilids and muricids with respect to the anatomy (Kantor, 1995; Richter & Luque, in press), reproductive strategy (parental care: Ponder, 1973; Robertson, 1976; sex change: Richter & Luque, in press) and protoconch (Richter & Thorson, 1975; Robertson, 1976; Scheltema & Williams, 1983; Riedl, 2000), an independent phylogenetic analysis using these characters should be undertaken in order to contrast it with the results of the analysis based on molecular characters carried out by Oliverio

& MARIOTTINI (2001 a). With this in mind, the present paper reviews the available information about feeding, anatomy, sexual strategy, parental care and protoconch of Coralliophilidae and carries out a cladistic analysis. Results are discussed and useful research lines are pointed out.

METHODS

A cladistic analysis with 25 selected characters and 55 characters states (see Appendix 1) was performed, from own and bibliographic data. The Hennig86 programme was used in order to infer a most parsimonious hypothesis of the phylogenetic relationships among 7 coralliophilid and 18 muricid species with the available database (see Appendix 2). The states of multistate characters were left unordered and the outgroup criterion was used to determine the ancestral state of each character. Two buccinoideans (*Nassarius vibex* and *Buccinum undatum*) were selected as outgroup. The analysis yielded 30 equally parsimonious trees from which a single consensus tree with 43 steps (ci 67; ri 85) was derived and discussed.

RESULTS AND DISCUSSION

Feeding

The anthozoans as food resource have been successfully exploited by coralliophilids, since they feed on seven anthozoan orders, namely Actiniaria, Scleractinia, Zoanthidea, Corallimorpharia, Antipatharia, Alcyonacea and Gorgonacea (Tab. 1). The preferred order of anthozoans is the Scleractinia, on which ca. 70% of the Recent species with known ecology and belonging to 7 genera feed. The degree of prey/host dependence varies widely within the family, which includes from ectobiotic mobile euryphagous species that feed on more than one order of anthozoans to endobiotic sessile stenophagous species which lack operculum and live embedded in the skeleton of hermatypic corals. Stenophagous coralliophilids, either ectobiotic or endobiotic, feeding only on one order of anthozoans, make about 85% of the species, and four genera are host specific (Tab. 1). The latter include the genera *Leptoconchus* and *Magilus*, whose species live exclusively associated to scleractinians corals, and *Rapa* and *Rhizochilus*, which are only found on soft corals (Alcyonacea) (LORENZ, 1996; OKUTANI, 2000) and black corals (Antipatharia) (KAY, 1979; POORMAN, 1981; KOSUGE & SUZUKI, 1985; OKUTANI, 2000), respectively. In stenophagous species, the shell shape is usually adapted to the anthozoan host on which they feed. This is the case of a few species that live attached to the surface of gorgonians (ALBERGONI & SPADA, 1972; POPPE & GOTTO, 1991), black corals (POORMAN, 1981; KOSUGE & SUZUKI, 1985) or massive hermatypic corals (MAES, 1967), or of those living buried inside the skeleton of hermatypic corals (MASSIN, 1982). However, adaptation of the shell to the host might also occur in euryphagous species, like for example in *Coralliophila meyendorffii* (OLIVERIO, 1989 a; OLIVERIO & MARIOTTINI, 2001 b).

Some associations of Recent coralliophilids with corals are very ancient in origin, dating back to the Paleogene. Endobiotic *Leptoconchus* species living inside the skeleton of faviid corals have existed at least since the upper Oligocene, and the association between the epibiotic *Galeropsis* (= *Quoyula*) species and Pocilloporidae at least dates back to the Lower Miocene (LOZOUET & RENARD, 1998). A fossil species of *Coralliophila* from the Lower Oligocene has been also found living endobiotically inside *Cladocora* (Faviidae) (LOZOUET & RENARD, 1998). Due to poor fossilization, associations of coralliophilids with anthipatarians or gorgonaceans are unknown. Anthipatarians apparently arose during the Miocene (WELLS & HILL, 1956), hence the association of coralliophilids and black corals might be more recent than those between endobiotic coralliophilids and hermatypic corals. As gorgonaceans have existed since the Cretaceous (BAYER, 1956), the association of coralliophilids and gorgonians might have arisen earlier than that with antipatharians. When the associations between actiniids, zoanthids, corallimorpharians and soft corals appeared is almost impossible to determine since these anthozoans lack any type of skeleton or have a skeleton formed by loose spicules embedded in a fleshy cenenchime, and do not fossilise easily.

The specialized anatomical and biological features of coralliophilids (see below) and the long lasting (presumably more than 38 my) association with anthozoans suggest that such characters may reflect adaptations to their particular mode of life. It would be therefore interesting to find out whether coralliophilids feeding on a particular group of anthozoans show specific and common adaptations, and if so, whether these particular adaptations have evolved independently as a result of convergent evolution in species feeding on the same group of anthozoans or whether they have evolved in a single evolutionary line within coralliophilids. This should be best analysed in the framework of a phylogenetic study using anatomical, reproductive, developmental or even molecular characters, in which food type should then be plotted on the resulting tree topology. The results of such an analysis would show whether food type implies or not phylogenetic affinity among species or genera or, in other words, if there is a coevolution between coralliophilids and anthozoans.

Anatomy

Coralliophilids are characterised by the lack of jaws and radula (THIELE, 1929; GOHAR & SOLIMAN, 1963; WARD, 1965; PONDER, 1973; MASSIN, 1987, 1990; KANTOR, 1995), and by a more or less long pleurembolic proboscis that acts as a sucking bomb and is adapted to penetrate, predigest and suck the soft tissue of their preys (WARD, 1965; KANTOR, 1995). Further features, such as the absence of accessory salivary glands, whose presence is considered a synapomorphy of neogastropods (PONDER, 1973; HARASEWYCH, 1984; TAYLOR & MORRIS, 1988; KANTOR, 1996; but see PONDER & LINDBERG, 1997), or the absence of dorsal glandular folds of the oesophagus and the fusion of the paired salivary ducts into a single duct are considered as characteristic for the group by KANTOR (1995, 1996), who assumed coralliophilids to be uniform at least in respect to the alimentary tract. However, the loss of accessory salivary glands is a tendency in the evolution of Neogastropoda and occurs in many groups, including Muricidae (WU, 1973; KOOL, 1993 a), Buccinoidea, Vasidae, Harpidae, Mitridae, Columbarii-

Table 1. Anthozoan preys of Coralliophilidae. **Abbreviations: A**, Antipatharia; **Ac**, Actiniaria; **Al**, Alcyonaria; **C**, Corallimorpharia; **G**, Gorgonacea; **S**, Scleractinia; **Z**, Zoanthidea.

Species	S	G	C	A	Ac	Z	Al	References
Babelomurex benoiti (Tiberi, 1855)							+	Barrajón, pers. com.
Babelomurex cariniferus (Sowerby, 1834)	+							Spada, 1968; Ghisotti & Spada, 1970; Chemello, 1986; Richter & Luque, in press
Babelomurex fearnleyi (Emerson & D'Attilio, 1965)	+							Okutani, 2000
Babelomurex hindsi (Carpenter, 1857)	+							Glynn & Wellington, 1983
Babelomurex oldroydi (Oldroyd, 1929)			+[1]					Wicksten & Wright, 1993
Coralliobia cumingii (H. & A. Adams, 1863)				+				D'Attilio & Kosuge, 1988
Coralliobia fimbriata (A. Adams, 1854)	+							Okutani, 2000
Coralliophila abbreviata (Lamarck, 1816)	+		+			+		Ward, 1965; Ott & Lewis, 1972; Miller, 1972, 1981; Wells & Lalli, 1977; Hayes, 1990
Coralliophila adansoni (Kosuge & Fernandes, 1989)							+	Rolán & Fernandes, 1990
Coralliophila brevis (Blainville, 1832)		+						Albergoni & Spada, 1969, 1972; Sabelli & Spada, 1980; Poppe & Goto, 1991; Richter & Luque, in press
Coralliophila bulbiformis (Conrad, 1837)	+							Kosuge & Suzuki, 1985; Okutani, 2000
Coralliophila caribaea Abbott, 1958	+	+	+			+		Miller, 1972, 1981; Wells & Lalli, 1977
Coralliophila clathrata (A. Adams, 1854)						+		Robertson, 1981; Rivas & Jay, 1996
Coralliophila costularis (Lamarck, 1816)	+							Kosuge & Suzuki, 1985; Lorenz, 1996; Okutani, 2000
Coralliophila erosa (Röding, 1798)	+							Kay, 1979; Kosuge & Suzuki, 1985; Drivas & Jay, 1996; Okutani, 2000
Coralliophila jeffreysii E. A. Smith, 1879	+							Okutani, 2000
Coralliophila kaofitorum Vega, Vega & Luque, 2002				+				Vega, Vega & Luque, 2002
Coralliophila meyendorffii (Calcara, 1845)	+				+			Garavelli & Melone, 1968; Spada, Sabelli & Morandi, 1973; Spada, 1979; Chemello, 1986; Luque, 1986; Oliverio, 1989 b; Pérez y Moreno, 1991; García-Raso et al., 1992; Chintiroglou & Koukouras, 1992
Coralliophila morishimai Kuroda & Shikama in Shikama, 1966		+						Kosuge & Suzuki, 1985
Coralliophila neritoidea (Lamarck, 1816)	+							Maes, 1967; Robertson, 1970; Kay, 1979; Kosuge & Suzuki, 1985; Soong & Chen, 1991; Lin & Liu, 1995; Lorenz, 1996; Drivas & Jay, 1996
Coralliophila panormitana (Monterosato, 1869)		+				+		Oliverio, 1989 a; Templado *et al.*, 1993
Coralliophila radula (A. Adams, 1855)	+							Drivas & Jay, 1996
Coralliophila richardi (Fischer, 1882)	+							Cecalupo, 1984; Bouchet & Warén, 1985; Luque, pers. obs.
Coralliophila squamosa (Bivona, 1838)					+			Garavelli & Melone, 1968 (as *C. lamellosa*); Oliverio, 1989 b
Coralliophila squamosissima (E. A. Smith, 1876)					+			Kosuge & Suzuki, 1985; Okutani, 2000
Galeropsis madreporarum (Sowerby, 1832)	+							Ghisotti, 1968; Keen, 1971; Glynn, Stewart & Mc Copker, 1983; Kay, 1979; Guzmán, 1988; Lozouet & Renard, 1998; Okutani, 2000
Leptoconchus cumingii Deshayes, 1863	+							Gohar & Soliman, 1963; Massin, 1982
Leptoconchus cyphastreae Massin, 1983	+							Massin, 1983
Leptoconchus expolitus Shikama, 1963	+							Massin, 1982; Okutani, 2000 (as *Magilus expolitus*)
Leptoconchus lamarckii Deshayes, 1863	+							Gohar & Soliman, 1963; Massin, 1982; Okutani, 2000
Leptoconchus peronii (Lamarck, 1818)	+							Gohar & Soliman, 1963 (as *L. globosus*); Massin, 1982 (as *L. striatus*), 1990; Okutani, 2000 (as *L. striatus*)
Leptoconchus rostratus A. Adams, 1864	+							Massin, 1982
Leptoconchus vangoethemi Massin, 1983	+							Massin, 1983
Magilus antiquus Montfort, 1810	+							Lamy, 1923; Massin, 1982; Drivas & Jay, 1996; Okutani, 2000
Rapa incurva (Dunker, 1853)							+	Okutani, 2000
Rapa rapa (Linnaeus, 1758)							+	Lorenz, 1996; Okutani, 2000
Reliquiaecava robillardi (Lienard, 1870)	+							Massin, 1987
Rhizochilus anthipatum Steenstrup, 1850				+				Kay, 1979; Kosuge & Suzuki, 1985; Okutani, 2000
Rhizochilus sp.				+				Poorman, 1981

Notes: [1] Under laboratory conditions, it is uncertain if it is a prey under natural conditions.

dae and Marginellidae (PONDER, 1973). Besides, a preliminary study on Mediterranean coralliophilids reveals that the complete reduction of the accessory salivary glands is not the rule in Coralliophilidae (pers. obs.). The fusion of the ducts of the salivary glands and the loss of the dorsal glandular folds of the oesophagus also needs to be confirmed in other species. The first character occurs at least in *Coralliophila abbreviata* (WARD, 1965), *Babelomurex naskensis* and *Babelomurex sentix* (KANTOR, 1995), and *Coralliophila meyendorffii* and *Babelomurex cariniferus* (pers. obs.). The loss of the dorsal glandular folds of the oesophagus occurs in *Coralliophila abbreviata* (WARD, 1965) and in *Babelomurex naskensis* and *Babelomurex sentix* (KANTOR, 1995).

While at present the presumed uniformity of the feeding apparatus awaits for confirmation, the anatomical and histological organisation of the reproductive system is certainly quite variable within the group and is useful in establishing internal relationships within coralliophilids, as will be shown later. On the basis of available information on the reproductive system of four Mediterranean species, viz. *Coralliophila squamosa* (Bivona, 1838) (OEHLMANN, 1994), *Coralliophila meyendorffii* (Calcara, 1845), *Coralliophila brevis* (Blainville, 1832) and *Babelomurex cariniferus* (Sowerby, 1834) (RICHTER & LUQUE, in press) and three Red Sea species, *Leptoconchus peronii* (= *globosus*) (Lamarck, 1818), *Leptoconchus cumingii* Deshayes, 1863 and *Magilopsis lamarckii* Deshayes, 1863 (GOHAR & SOLIMAN, 1963, included by MASSIN, 1982 in *Leptoconchus*), four basic types of reproductive system organisations within coralliophilids can be recognised. They differ from each other in the presence or absence of a gonopericardial duct, seminal receptacle (or sperm ingesting gland) or a slit in the proximal region of the prostata, in the secretory areas of the capsule gland, in the structure of the bursa copulatrix, in the grade of closure and the number of folds of the ventral channel, and in the shape of the penis and the albumen gland. Within the genus *Coralliophila* two of these four types occur, one shared by *Coralliophila meyendorffii* and *C. brevis*, while the other corresponds to *C. squamosa*. The reproductive system of the latter species resembles more closely to that of certain Ocenebrinae than to any of the other known coralliophilids, suggesting that the genus *Coralliophila* is polyphyletic. The third type of reproductive system is represented by the genus *Leptoconchus*, which apparently lack a proximal seminal receptacle (RICHTER & LUQUE, in press). Finally, the fourth type corresponds to *Babelomurex cariniferus*, whose reproductive system is similar to that of *Coralliophila meyendorffii* and *C. brevis*, but dif-

Table 2. Data on reproductive and life history strategy of coralliophilids. **Abbreviations: A**, aggregates; **b**, both sexes mobile; **D**, mode of development; **f**, females; **m**, males; **M**, mobility; **p**, planktotrophic; **PCF**, positive correlation between female fecundity and female size; **PF**, pseudopenis in females; **s**, sessile; **ss**, semisessile; **SSD**, sexual size dimorphism; **SR**, sex ratio; **v**, variable; +: presence of the character; ? no data available.

Species	SR (m:f)	SSD	PF	A	PCF	M	D	References
Babelomurex cariniferus	1:1[1]	+	+	?	+	b	p	RICHTER & LUQUE, in press
Coralliophila abbreviata	v	+	+	+	+	b	p	WARD, 1965; WELLS & LALLI, 1977; HAYES, 1990; FIORONI, OEHLMANN & STROBEN, 1991
Coralliophila brevis	?	?[4]	+	?	?	ss[6]	?	ALBERGONI & SPADA, 1972; RICHTER & LUQUE, in press
Coralliophila caribaea	1:1[1]	+	?	?	+	b	p	WELLS & LALLI, 1977
Coralliophila meyendorffii	1:1[1]	+	+	?	+	b	p	RICHTER & LUQUE, in press
Coralliophila neritoidea	>1[2]	+	+	+	+	b	p	SOONG & CHEN, 1991; LIN & LIU, 1995 (both references as *C. violacea*)
Coralliophila squamosa	?	?4	+	?	?	b	?	OEHLMANN, 1994 (as *Coralliophila lamellosa*)
Leptoconchus cyphastreae	?[8]	?	?	?	?	f: s, m: ?	?	MASSIN, 1983
Leptoconchus cumingii	?	?	?	?	?	s	p	GOHAR & SOLIMAN, 1963
Leptoconchus lamarckii	?	?	?	?	?	s	p	GOHAR & SOLIMAN, 1963 (as *Magilopsis lamarckii*)
Leptoconchus peronii	1:1[3]	+	?[5]	?	?	f: s, m: ss[7]	p	GOHAR & SOLIMAN, 1963 (as *L. globosus*); MASSIN, 1982 (as *L. striatus*), 1990
Leptoconchus vangoethemi	?[8]	?	?	?	?	f: s, m: ?	?	MASSIN, 1983
Reliquiaecava robillardi	?[8]	?	?	?	?	f: s, m: ss/s	p	MASSIN, 1987
Rhizochilus sp.	?	?	?	+	?	s	p	POORMAN, 1981

Notes: [1] Sex ratio does not deviate significantly from the expected 1:1 Fischer sex ratio for dioic species.

[2] Males are significantly more abundant than females.

[3] MASSIN (1990) did not reported about the sex ratio of the species, but a goodness of fit test performed with the frequency data reported in the paper reveals that male and female proportion did not depart significantly from the expected 1:1 ratio for dioic species.

[4] Sample size was too small to test statistically sexual size dimorphism. Nevertheless, the smallest individual/s of the sample was/were males, suggesting the existence of sexual size dimorphism.

[5] MASSIN (1990) observed a couple of males with vestigial penis, but since gonads were not examined microscopically, it cannot be ruled out that the individuals were in fact females. At least, the vestigial penis suggested that individuals might reduce penis as a consequence of sex change from male to female.

[6] According to ALBERGONI & SPADA (1972) large individuals of *C. brevis* tend to attach firmly to the gorgonian on which they feed, while smaller individuals are mobile.

[7] MASSIN (1982, as *L. striatus*) observed free living individuals of about 3 mm in shell length, which fall within the size range of males according to MASSIN (1990). Since MASSIN (1990) also observed males burrowed inside the coral skeleton, this means that males pass through a creeping stage before being sessile.

[8] Males are virtually absent in the samples.

fers in the shape of the albumen gland and the penis, and in the number of folds and grade of closure of the ventral channel (RICHTER & LUQUE, in press). The common features of the reproductive system of these three species include among others the reduction of the dorsal lobe of the capsule gland and the existence of a large vestibule, both of which are potentially synapomorphies of a subgroup within coralliophilids, as will be discussed later. Whether the genus *Leptoconchus* and *Coralliophila squamosa* also presents these characters is still unclear and should be found out. Apparently, at least at the present stage of knowledge, there is no character of the reproductive system common for all coralliophilids except for the structure of the penis duct. This might be rather a consequence of the different degree of detail of the anatomical studies. More thorough anatomical studies on the reproductive system of additional species from each of these genera and of other genera not used in the analysis should be undertaken in order to define genera and to unravel relationships within coralliophilids.

Reproductive strategy

Although coralliophilids have been traditionally considered as dioic species (ROBERTSON, 1970; OLIVERIO, 1989 b), there are up to date no direct (histological) nor indirect evidences supporting this assumption. By contrast, the relatively high incidence of reduced penis in females of coralliophilids and the widespread sexual size dimorphism with males smaller than females rather points to the existence of protandry in coralliophilids, which has been definitely proved to occur in *C. meyendorffii* and *B. cariniferus* by the results of a laboratory monitoring of penis reduction coupled with an histological and anatomical study of the reproductive system of monitored individuals (RICHTER & LUQUE, in press). Other life history traits of coralliophilids, such as the tendency to form aggregates, sessility and dependence on a spatially discontinuously distributed food source have been linked in other prosobranchs to a special type of hermaphroditism called environmental sex determination (ESD). ESD has been observed in calyptraeids (HOAGLAND, 1978), eulimids (WARÉN, 1980, 1983) and giant territorrial limpets (WRIGHT, 1989). Departures from the expected 1:1 sex ratio for dioecia, which are common in protandric species, are also found in coralliophilids. Samples with a very low proportion of males or with virtually absent males are common in coral boring species (MASSIN, 1983, 1987). This also occurs in sedentary protandric gastropods with a mobile male phase, like the sedentary turritellid *Vermicularia spirata* (BIELER & HADFIELD, 1990) and the non-gregarious *Crepidula dilatata* (GALLARDO, 1976). In *Coralliophila neritoides* (Lamarck, 1816) the sex ratio is skewed toward males (SOONG & CHEN, 1991), as is the rule in protandric species. In *Coralliophila abbreviata* (Lamarck, 1816) the sex ratio is variable depending on the geographical locality, and some populations have equal proportion of males and females, while in others males predominates (WELLS & LALLI, 1977; HAYES, 1989).

WARNER, ROBERTSON & LEIGH (1975) linked the correlation between fecundity and size or age to the sexual strategy of the species. According to the authors, if the fecundity of a sex increases with size or age, sex change is advantageous over dioecia. In coralliophilids usually exists a positive correlation between female fecundity and female size. Hence, according to the hypothesis of WARNER, ROBERTSON & LEIGH (1975) protandry should be expected. An increase of female fecundity with female size has been reported *in C. neritoidea* (LIN & LIU, 1995), *C. abbreviata* and *C. caribaea* (WELLS & LALLI, 1977) and also occur in *C. meyendorffii* and *B. cariniferus* (RICHTER & LUQUE, unpublished).

Table 2 summarises the available information about aspects of coralliophilid biology that has been related to protandry or ESD in other gastropods. Except for *Coralliophila meyendorffii* and *Babelomurex cariniferus*, in most of the species the sexual strategy has still to be assessed by using histological methods together with a field or laboratory monitoring of penis reduction (= sex change). This monitoring must follow an experimental design in order to test dependence of percentage of individuals reducing penis (= changing sex) on initial population structure, as such undertaken for calyptraeids by HOAGLAND (1978) and COLLIN (2000) and for coralliophilids by RICHTER & LUQUE (in press). In *Coralliophila meyendorffii* and *Babelomurex cariniferus* the evidences pointing to protandry includes direct observation of penis reduction, the existence of transitional sexual stages close to onset and during breeding season and sexual size dimorphism with males smaller than females (RICHTER & LUQUE, in press). In *Coralliophila neritoides* an environmental sex determination has been proposed (SOONG & CHEN, 1991), although it awaits for histological confirmation. In this species evidences pointing to ESD are sexual size dimorphism with males smaller than females, a skewed sex ratio toward males, the observed degeneration of the penis and the correlation of the smallest female size and the largest male size to aggregates structure. In *Coralliophila squamosa*, pseudohermaphroditism has been reported (FIORONI, OEHLMANN & STROBEN, 1991; OEHLMANN, 1994). However, this statement has to be checked because this conclusion is based on the observation of four females with pseudopenis histologically similar to male penis in a sample of six individuals (4 females: 2 males). Such fact does not rule out the existence of protandry.

Parental care

Contrary to most neogastropods, which lay benthic egg capsules, coralliophilids incubate their brood inside the female mantle cavity enclosed in membranous flat elliptical egg-pouches (GOHAR & SOLIMAN, 1963; GHISOTTI & SPADA, 1970; WELLS & LALLI, 1977; ROBERTSON, 1980; MASSIN, 1983, 1987, 1990; ROLÁN & FERNANDES, 1990; LIN & LIU, 1995; RICHTER & LUQUE, in press). Brooding mechanism, however, is not the same in all species. While *Coralliophila abbreviata*, *C. neritoides* and species of *Leptoconchus* breed loose unattached egg-capsules inside the pallial cavity until larvae are mature and ready to hatch, females of *Coralliophila caribaea* push the capsules they breed outside the pallial cavity while development proceeds, and attach them to a groove between foot and operculum (WELLS & LALLI, 1977). Besides, in *Leptoconchus* (GOHAR & SOLIMAN, 1963), *Coralliophila meyendorffii* and *Babelomurex cariniferus* (pers.

Table 3. Available data on protoconch, larval development, and bathymetrical and geographical distribution of coralliophilids. Data with ? are not included in the statistical test. **Abbreviations: AD**, area of distribution: **A**, Atlantic; **AA**, amphi-Atlantic; **EA**, East Atlantic; **EP**, East Pacific; **I**, Indian Ocean; **IP**, Indo-Pacific; **M**, Mediterranean; **RS**, Red Sea; **P**, Pacific Ocean; **WP**, West Pacific; **BR**, bathymetric range (exact depths in meters): **b**, bathyal; **dw**, deep-water; **s**, sublittoral; **sm**, seamounts; **sw**, shallow-water; **MD**, mode of development: **p**, planktotrophic; **np**, non planktotrophic; * inferred from protoconch; ** number of whorls of protoconch correspond to planktotrophic type, but lack of shell ornamentation points to non-planktotrophic development, no reliable inference can be made; *** number of whorls indicates non-planktotrophic development, but shell ornamentation points to planktotrophic one, no reliable inference can be made; **?** inferred type of larval development doubtful, since shell ornamentation is eroded; **NW**, number of whorls of protoconch; **P**, type of protoconch, according numbers giving in text: **e**, protoconch completely or partially eroded; – no data available.

Species	P	NW	MD	BR	AD	References
Babelomurex cariniferus (Sowerby, 1834)	7[1]	1.5	p	s	EA, M	D'Attilio, 1972; García-Talavera, 1983; Kosuge & Fernandes, 1988; Richter & Luque, in press
Babelomurex cariniferoides (Shikama, 1966)	1	3	p*	-	WP	Kosuge, 1986 a; Okutani, 2000
Babelomurex centimanus Kosuge, 1985	2	3	p*	dw	WP	Kosuge, 1985 b; Kosuge & Suzuki, 1985
Babelomurex cookae Kosuge, 1988	1	2.5	p*	135-315	EP	Kosuge, 1988 a
Babelomurex deburghiae (Reeve, 1857)	5	1.5-2	np?	20-200	WP	Kosuge, 1986 a; Okutani, 2000
Babelomurex fusiformis (Martens, 1902)	e	2	np?	486	IP	Azuma, 1973; Kosuge & Suzuki, 1985
Babelomurex glaber Kosuge, 1998	7	2	np*	490	I	Kosuge, 1998
Babelomurex hirasei (Shikama, 1964)	7	2	np*	-	WP	Kosuge, 1986 a, d; Okutani, 2000
Babelomurex lischkeanus (Dunker, 1882)	3	3	**	-	I, WP	D'Attilio, 1972 (as *Latiaxis lischkeana*); Kosuge & Suzuki, 1985; Kosuge, 1986 c
Babelomurex memimarumai Kosuge, 1985	6	1	***	dw	WP	Kosuge, 1985 b
Babelomurex miyokoae Kosuge, 1985	1	2.5	p*	-	WP	Kosuge, 1985 b
Babelomurex squalida Kosuge, 1985	1	2	p*	-	WP	Kosuge, 1985 b
Babelomurex stenospinus (Kuroda, 1961)	5	2	p*	30-200	WP	Kosuge, 1986 a; Okutani, 2000
Babelomurex yamatoensis Kosuge, 1986	4	2	p*	dw	WP	Kosuge, 1986 a; Okutani, 2000
Babelomurex yumimarumai Kosuge, 1985	4	2	p*	dw	WP	Kosuge, 1985 b; Kosuge & Suzuki, 1985
Coralliophila abbreviata (Lamarck, 1816)	-	-	p	sw, sm	WA	Bandel, 1975; Wells & Lalli, 1977
Coralliophila aberrans (C. B. Adams, 1850)	1	4.5	p	sw, sm	WA	Bandel, 1975; Leal, 1991
Coralliophila adansoni (Kosuge & Fernandes, 1989)	e	1.5	np?	sw	EA	Kosuge & Fernandes, 1989 (as *Ocinebrina adansoni*); Rolán & Fernandes, 1990
Coralliophila caribaea Abbott, 1958	1	4.5	p	sw	WA	Bandel, 1975; Wells & Lalli, 1977; Cosel, 1982; Jong & Coomans, 1988; Leal, 1991
Coralliophila carnosa Kosuge, 1986	1	3-4	p*	sw	WP	Kosuge, 1986 d; Okutani, 2000
Coralliophila clathrata (A. Adams, 1854)	1	ca. 4	p	sw	IP	Yen, 1935; Robertson, 1980; Kosuge & Suzuki, 1985
Coralliophila flava Kosuge, 1985	4	2	p*	-	I, WP	Kosuge, 1985 b
Coralliophila kaofitorum Vega, Vega & Luque, 2002	1	3.5-4	p*	18-48	EA	Vega, Vega & Luque, 2002
Coralliophila leucostoma Kosuge, 1986	e	3	p*	sw	WP	Kosuge, 1986 b
Coralliophila liltvedi Kosuge, 1986	1	3	p*	245	EA	Kosuge, 1986 b
Coralliophila meyendorffii (Calcara, 1845)	1	4.25	p	s	EA, M	Richter & Thorson, 1976; Cosel, 1982; Richter & Luque, in press
Coralliophila mitraeforma Kosuge, 1985	1	2.5	p*	dw	WP	Kosuge, 1985 b; Okutani, 2000
Coralliophila occidentale Kosuge & Fernandes, 1988	1	4	p*	60	EA	Kosuge & Fernandes, 1988
Coralliophila ohmurai Kosuge, 1985	4	2	p*	12	WP	Kosuge, 1985 b
Coralliophila raramaculatus Kosuge & Fernandes, 1989	e	2.5	p?	1	EA	Kosuge, 1989
Coralliophila richardi (P. Fischer, 1882)	1	4	p*	b	AA, M	Cecalupo, 1984; Taviani & Taviani, 1986; Bouchet & Warén, 1985
Coralliophila roseocephala Kosuge, 1986	1	3-4	p*	200	WP	Kosuge, 1986 d; Okutani, 2000
Coralliophila tetragona Kosuge, 1986	e	4	p*	-	I	Kosuge, 1986 b
Galeropsis madreporarum (Sowerby, 1832)	1	4	p	sw	IP	Scheltema & Williams, 1983
Hirtomurex nakamurai Kosuge, 1985	7	2	np*	150	WP	Kosuge, 1985 a; Okutani, 2000
Hirtomurex oyamai Kosuge, 1985	e	2	np?	180	WP	Kosuge, 1985 a
Hirtomurex vertigo Kosuge, 1986	e	3	p?	120	WP	Kosuge, 1986 d
Latiaxis latipinnatus Azuma 1961	1	3	p*	-	WP	Kosuge & Suzuki, 1985; Kosuge, 1986 a
Leptoconchus cumingii Deshayes, 1863	-	-	p	sw	RS, I	Gohar & Soliman, 1963; Massin, 1982
Leptoconchus lamarckii (Deshayes, 1863)	-	-	p	sw	RS, I, P	Gohar & Soliman, 1963; Massin, 1982; Okutani, 2000
Leptoconchus peronii (Lamarck, 1818)	-	-	p	sw	RS, I, P	Gohar & Soliman, 1963 (as *L. globosus*); Massin, 1983 (as *L. striatus*), 1990; Okutani, 2000 (as *L. striatus*)
Mipus basicostatus Kosuge, 1988	1	ca. 3	p*	-	I	Kosuge, 1988 b
Mipus hotei Kosuge, 1985	4	2	p*	160–190	WP	Kosuge, 1985 b; Okutani, 2000
Mipus intermedius Kosuge, 1985	1	3	p*	-	WP	Kosuge, 1985 b; Okutani, 2000
Mipus ovoideus Kosuge, 1985	7	2	p*	-	WP	Kosuge, 1985 b; Okutani, 2000
Mipus eugeniae (Bernardi, 1853)	7	2	np*	-	WP	D'Attilio, 1972; Kosuge & Suzuki, 1985
Rapa rapa (Linnaeus, 1758)	1	ca. 3	p*	sw	I, P	D'Attilio, 1972 (as *Rapa papyracea*); Kosuge & Suzuki, 1985
Reliquiaecava robillardi (Lienard, 1870)	e	3	p*	sw	I, P	Massin, 1982, 1987
Rhizochilus sp.	1	3	p*	dw	EP	Poorman, 1981

Notes: [1] D'Attilio (1972) described a smooth and globose protoconch of 1½ whorls for *Babelomurex babelis* (a junior synonym of *B. cariniferus*), but our own observations prove that planktotrophic veligers hatched from egg-capsules.

obs.) the capsules with larvae ready to hatch are then freed outside the mantle cavity while in *C. caribaea* they remain attached to the foot through a filament. Differences in the breeding mechanism of gastropods exhibiting brood protection are usual. Vermetid females incubate egg-capsules loose inside the pallial cavity, or attached to the inner surface of the vermiform shell. The type of brooding is linked to the presence or absence of a slit in the mantle (MORTON, 1965), but it has little phylogenetic value in vermetids, since in many genera both types of incubation exists (HADFIELD, KAY, GILLETTE & LLOYD, 1972; CALVO, 1999). *Coralliophila meyendorffii* and *Babelomurex babelis*, which show the same breeding behaviour (unattached capsules) than *Coralliophila abbreviata* and *Leptoconchus* lack a ventral pedal gland (RICHTER & LUQUE, in press). In *Coralliophila caribaea*, the presence of a filament fixing the egg-capsules to the foot might indicate to the contrary the existence of a ventral pedal gland, since in Neogastropods the formation of a stalk in the capsule is linked to a moulding and fixing process carried out by the ventral pedal gland (ANKEL, 1936). If brooding type in coralliophilids is related to the presence or absence of a pedal gland it might be a good systematic criteria, since the loss of a pedal gland is an evolutionary step. Whether more coralliophilid species exhibit the same breeding mechanism than *Coralliophila caribaea*, and whether this is linked to the existence of a ventral pedal gland should be find out. If, in fact, a ventral pedal gland exists, its presence should be regarded as a primitive character, and its absence as a secondary loss.

Brood care has been also reported in volutids (MARCHE-MARCHAD, 1968, 1977, 1980; PENCHASZADEH & DE MAHIEU, 1976; KNUDSEN, 1993) and buccinids (HUGHES, 1986), but in these groups females breed the egg-capsules in the ventral pedal gland. Therefore, within the frame of neogastropod phylogeny, brooding inside the pallial cavity could be considered as a synapomorphy of coralliophilids. However, as discussed later on, brooding can be only considered as a synapomorphy of a subgroup of coralliophilids, because its existence remains unknown in most species, and particularly in key species like *Coralliophila squamosa*, which, as suggested by RICHTER & LUQUE (in press) and supported by the present phylogenetic analysis, might represent a primitive coralliophilid with a primitive organisation of the reproductive system.

Protoconch and larval development

The protoconch of coralliophilids is unknown in most of species, because it is usually lacking or eroded in adult and even young specimens. In some genera, the protoconch of a single or a few species have been studied. Nevertheless, a protoconch with a multispiral larval shell with sinusigerous lip and strong knobbed spiral cords crossed by axial ribs has been considered to be diagnostic for coralliophilids (ROBERTSON, 1976; RICHTER & THORSON, 1975; SCHELTEMA & WILLIAMS, 1983; RIEDL, 2000). Most of recent species in which the protoconch is known presents such a type of larval shell (D'ATTILIO, 1972; RICHTER & THORSON, 1975; ROBERTSON, 1976, 1980; SCHELTEMA & WILLIAMS, 1983; TAVIANI & TAVIANI, 1986; LEAL, 1991; RIEDL, 2000; VEGA, VEGA & LUQUE, 2002), which indicates a planktotrophic development and appears in the fossil record at the Middle Eocene (LOZOUET & RENARD, 1998; RIEDL, 2000). However, D'ATTILIO (1972) and KOSUGE (1985 a, b; 1986 a, d; 1998) reveal a much wider diversity of protoconchs, that also include globose, paucispiral and smooth larval shells indicating a non-planktotrophic development.

Table 3 lists species whose protoconch has been described. Each protoconch with preserved microsculpture is classified morphologically according to the number of whorls and microsculpture and designated with a number. Correlation between number and protoconch morphology is as follow:

1) multispiral (more than 2 whorls) with 2 spiral cords and axial ribs.

2) multispiral (more than 2 whorls) with a single spiral thread

3) multispiral (more than 2 whorls) globose and smooth.

4) paucispiral (up to 2 whorls) with spiral cords and axial ribs.

5) paucispiral (up to 2 whorls) with faint spiral sculpture.

6) paucispiral (up to 2 whorls) with faint axial sculpture.

7) paucispiral (up to 2 whorls) globose and smooth.

Data on bathymetric range, geographic distribution and type of larval development are also indicated. In most species mode of development has not been directly observed and is here inferred from morphological characters of protoconch. Morphological types 1 and 2 correspond to planktotrophic, while type 7 to non-planktotrophic development. The modes of larval development that correspond to type 3, 4, 5 and 6 has still to be verified by studying the protoconch with SEM and culturing spawns until hatching of larvae.

The different types of protoconch suggests that it might be of taxonomic value in coralliophilids, at least at specific level. However, more information is needed in order to assess the value of protoconch morphology at supraspecific level. According to D'ATTILIO (1972), protoconch is not useful in defining genera, but might reflect zoogeographical boundaries. As shown in table 3, there is a relative high incidence of paucispiral smooth protoconchs in the Western Pacific when compared to the Atlantic basin, where at present no species with a paucispiral smooth protoconch has been described. In the Western Pacific 18% of the species (4 out of 22) bear paucispiral and smooth protoconch indicating non-planktotrophic development. These species belong to three genera (*Babelomurex*, *Hirtomurex* and *Mipus*), which also have plankototrophic species with multispiral axially and spirally ribbed protoconchs. Protoconch has also apparently ecological implications. A goodness of fit test reveals that the factor of number of whorls (paucispiral vs. multispiral) is significantly linked to depth range (deep sea vs. shallow waters vs deep sea-shallow waters) ($X^2 = 15.12$; $p < 0.001$; df= 2). Type of larval development also seems to depend on depth range ($X^2 = 52.6$; $p < 0.001$; df= 2), with non-planktotrophic development apparently restricted to deep sea localities (12%, 0% in shallow waters). It should be investigated whether the shift from planktotrophy to non-planktotrophy in Coralliophilidae is like in other caenogastropods an adaptative response to ecological factors or whether it is related to local historical fac-

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Nassarius vibex
Buccinum undatum
Coralliophila squamosa
Babelomurex cariniferus
Coralliophila brevis
Coralliophila meyendorffii
Leptoconchus lamarckii
Leptonconchus cumingii
Leptoconchus peronii
Ocenebra erinacea
Nucella lapillus
Phyllonotus trunculus
Bolinus brandaris
Nucella lamellosa
Paziella pazi
Trophon geversianus
Boreotrophon aculeatus
Morula papillosa
Rapana rapiformis
Concholepas concholepas
Dicathais orbita
Vasula melones
Nassa iopas
Pinaxia coronata
Drupa morum
Plicopurpura patula
Neorapana muricata

Figure 1. Strict consensus cladogram generated in the analysis.

tors (changes in sea level, water currents, temperature fluctuations during Late Pliocene- Pleistocene).

In spite of its ecological and biogeographical implications, protoconch might also reflect phylogenetic relationships in coralliophilids. The Western Pacific species of *Mipus* and *Hirtomurex* that have paucispiral and smooth protoconch and similar teleoconchs might have evolved from a common ancestor with planktotrophic development through a process that involves a transversal and subsequent allopatric speciation.

Phylogenetic implications of anatomical and reproductive characters

As pointed out in introduction, only recent phylogenetic analysis using molecular characters has been shown to be a good approach for unravelling the phylogenetic relationships of Coralliophilidae. HARASEWYCH *et al.* (1997) and OLIVERIO & MARIOTTINI (2001 a) demonstrated the close affinity between muricids and coralliophilids as suggested by their similarities in external anatomy and shell. The analysis of the latter authors, who compared sequences coding for 12S rDNA of five coralliophilid species representing five different coralliophilid genera and three muricids representing three subfamilies (Rapaninae, Muricinae and Ocenebrinae) also revealed the coralliophilids as a monophyletic clade within the Muricidae. The internal relationships of the coralliophilids, however, were only partly resolved. The different phylogenetic trees obtained only coincided in that the "spiny" group (*Babelomurex*, *Hirtomurex* and *Latiaxis*) was a monophyletic clade.

The present preliminary phylogenetic analysis based on anatomical characters of the reproductive and alimentary system, the reproductive strategy (sexuality, parental care) and larval development generates a strict consensus tree (Fig. 1) that reveals the Coralliophilidae as a monophyletic group characterised by the synapomorphy of protandry, assuming that, as suggested by a few evidences (see Tab. 2), *C. squamosa* and *Leptoconchus* species are protandric. The character state changes at each node are given in Appendix 3, corresponding to the nodes numbers on the cladogram in Fig. 1. Thus, the result supports the monophyly pointed out by the analysis of OLIVERIO & MARIOTTINI (2001 a), and also coincides with the pattern of the internal relationships of the Muricidae excluding Coralliophilidae obtained by these authors. Rapaninae appears in both analysis as the most derived subfamily of Muricidae, while an Ocenebrinae species, *Nucella lapillus*, represents together with the paraphyletic *Ocenebra erinacea* the less derived taxon of the Muricidae. *Phyllonotus trunculus*, a Muricinae species, is more derived than *Nucella lapillus* and less than Rapaninae. However, contrary to the phylogenetic hypothesis proposed by OLIVERIO & MARIOTTINI (2001 a), that holds that Coralliophilinae represents an evolutionary line within Muricidae, with the Rapaninae being a sister taxon of Coralliophilinae, the present analysis splits Coralliophilidae and Muricidae into two independent monophyletic clades. The monophyly of Muricidae is supported by the synapomorphies of presence of accessory boring organ (ABO), and presence of more or less developed right and left accessory salivary glands. The latter can be secondarily lost as occur in *Drupa* (WU, 1973; KOOL, 1993 a). However, if the key species *Coralliophila squamosa* would present an ABO and/or both accessory salivary glands, the monophyly of the Muricidae would no longer be sustained and the group would break down into various paraphyletic clades, while the coralliophilids excluding *C. squamosa* would keep their monophyly. One of the phylogenetic trees obtained by OLIVERIO & MARIOTTINI (2001 a), when including in their analysis four 12 S rDNA sequences of further four muricids species, also separates Coralliophilidae and Muricidae into two monophyletic independent clades. However, this result was rejected as the less plausible hypothesis by a maximum likelihood analysis. The present phylogenetic analysis is also congruent with HARASEWYCH (1984) in that Trophoninae represents a late offshoot of the Muricidae closely related to *Paziella pazi*, a Muricinae.

Concerning the internal relationships of the Coralliophilidae, the genus *Coralliophila* seems to be polyphyletic, as suggested by OLIVERIO & MARIOTTINI (2001 a), with a primitive species, *Coralliophila squamosa* branching off very early at the base of coralliophilids, and two derived species (*Coralliophila meyendorffii* and *C. brevis*) that appear grouped in a clade with *Leptoconchus* and *Babelomurex*. This clade of *Babelomurex-Leptoconchus-C. meyendorffii-C. brevis* is characterised by the synapomorphy of brood care in the mantle cavity, an often invoked diagnostic character for coralliophilids, and by other two characters that are homoplastic (absence of female gonopericardial duct and planktotrophic larval development). If brood care is confirmed in the primitive species *C. squamosa*, it should be considered as a synapomorphy for the whole group. The *Babelomurex-Leptoconchus-C. meyendorffii-C. brevis* clade is also defined by other four potential synapomorphies, namely the absence of a ventral pedal gland, the fusion of the salivary ducts into a single duct, a long vestibule and the reduction of the dorsal lobe of the capsule gland. All of them occur in *Babelomurex* and *C. meyendorffii* (RICHTER & LUQUE, in press; pers. obs.), but still has to be confirmed in *Leptoconchus* species. In *C. brevis* only the fusion of the salivary glands has to be confirmed. If the fusion of the salivary glands, the absence of a ventral pedal gland and a long vestibule are confirmed in *Leptoconchus* species, these characters turn out to be synapomorphies of the clade. If these characters are also present in *C. squamosa*, they should be considered as synapomorphies for the whole group of coralliophilids. The clade *Leptoconchus* is characterised by a few homoplastic characters which are shared with other muricids (i. e., absence of proximal sperm pouch) and by a small bursa copulatrix separated from the oviduct and with an independent opening, which is unique for *Leptoconchus*, while the clade *C. brevis-C. meyendorffii* is defined by the presence of a penial papilla, which is convergent with the penial papilla of *Buccinum undatum* and Trophoninae.

The analysis point out also that at present there is no autoapomorphy defining *C. squamosa*, which at the present state of knowledge probably share with the remaining coralliophilids only a reproductive strategy characterised by a sex change from male to female (see above and Tab. 2), and this has still to be corroborated by histological methods and field or laboratory monitoring.

From the above it becomes evident that phylogenetic analysis based on anatomical and reproductive characters is useful as an alternative approach for the establishment of relationships within coralliophilids and among coralliophilids and muricids. Nevertheless, still much work has to be done in that sense. Further phylogenetic analyses including more species and genera, specially key species with primitive characters (i. e., *C. squamosa*), and more information on anatomical and biological characters, in particular on characters that have been revealed in the present study as synapomorphies or potential synapomorphies, are necessary.

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Lavoro accettato l'11 Dicembre 2001

Appendix 1

Lists of characters and their states used for the phylogenetic analysis

Reproductive biology

0. Reproductive behaviour: 0= non-brooding; 1= brooding inside the pallial cavity.

Female reproductive system

1. Gonopericardial duct: 0= absent; 1= present.
2. Dorsal lobe of the capsule gland: 0= reduced; 1= well developed.
3. Anteroventral lobe of the capsule gland: 0= absent; 1= present.
4. Proximal sperm pouch (= seminal receptacle or sperm ingesting gland) between albumen gland and capsule gland: 0= absent; 1= present.
5. Posterior seminal receptacles: 0= absent; 1= connected to oviduct between albumen gland and sperm ingesting gland.
6. Seminal receptacle associated to albumen gland: 0= absent; 1= present.
7. Duct leading from the oviduct to the proximal sperm pouch: 0= absent; 1= lined by a ciliated epithelium; 2= lined by a smooth epithelium.
8. Vestibule: 0= vestibule short, does not extend appreciably beyond capsule gland; 1= vestibule long.
9. Bursa copulatrix: 0= continuous with capsule gland; 1= lateral diverticulum of anterior oviduct sharing a common genital pore with the oviduct 2= lateral diverticulum of anterior oviduct with an independent opening to the pallial cavity distinct from that of the oviduct

Male reproductive system

10. Gonopericardial duct: 0= absent; 1= reduced to a blind diverticulum; 2= present.
11. Prostata: 0= with proximal slit-like opening; 1= completely closed; 2= with a short muscular duct connecting pallial cavity with lumen.
12. Prostata: 0= without subepithelial gland cells; 1= with subepithelial gland cells.
13. Penis tip: 0= without papilla; 1= with papilla.
14. Vas deferens: 0= without subepithelial glandcells; 1= with subepithelial glandcells
15. Prostata: 0= with line of closure; 1= without line of closure.
16. Blind diverticulum from renal organ to sperm duct: 0= absent; 1= present.

Sexual strategy

17. Sexual strategy: 0= dioecia; 1= protandry or ESD.

Accessory structures for the performance of egg-capsules

18. Ventral pedal gland: 0= absent; 1 = present.

Alimentary system

19. Ducts of salivary glands: 0= one pair separated; 1= ducts fusing anteriorly in a single duct.
20. Accesory salivary glands: 0= absent; 1= both reduced to very short straight tubes; 2= one pair more or less developed with equal or unequal length.

Foot

21. Accesory boring organ: 0= absent; 1= present.

Mantle

22. Anal gland: 0= absent; 1= present.

Operculum

23. Operculum: 0= present; 1= absent.

Developmental traits

24. Larval development: 0= planktotrophic; 1= non-planktotrophic.

Appendix 2

Data matrix used for the phylogenetic analysis

Data compiled from Fretter (1941), Gohar & Soliman (1963), Harasewych (1984), Kool (1988, 1993 a, b), Amor (1990); Oehlmann (1994); DeMaintenon (2001), Richter & Luque (in press) unknown or not comparable.

Taxon	Matrix
Nassarius vibex (Say, 1822)	01?110010102101110100000?
Buccinum undatum Linnaeus, 1758	01111002?1?211??001000001
Trophon geversianus (Pallas, 1774)	0011000001001100001021101
Boreotrophon aculeatus (Watson, 1882)	0011000000001100001021101
Morula papillosa Schumacher, 1817	0??0110?00?11000001021100
Rapana rapiformis (Born, 1778)	0??1101?00?11000001021100
Concholepas concholepas (Bruguière, 1789)	0???101?00?11000001021100
Dicathais orbita Gmelin, 1791	0??1101?00?11000001021100
Vasula melones (Duclos, 1832)	0??1101?00?11000001021100
Nassa iopas H. & A. Adams, 1853	0??1101?00?11000001021100
Pinaxia coronata H. & A. Adams, 1853	0??1101?00?11000001021100
Drupa morum Röding, 1798	0??1101?00?11000001021100
Plicopurpura patula (Linnaeus, 1758)	0??1101200?11000001021100
Neorapana muricata (Broderip, 1832)	0???101?00?11000001021100
Phyllonotus trunculus (Linnaeus, 1758)	0111100??10000000010?1101
Bolinus brandaris (Linnaeus, 1758)	0111100??10000000010?1101
Ocenebra erinacea (Linnaeus, 1758)	0111100201101000001021101
Nucella lamellosa (Gmelin, 1791)	0011100?01001000001021101
Nucella lapillus (Linnaeus, 1758)	0111100201001000001021101
Babelomurex cariniferus (Sowerby, 1834)	1000100111001000010011?100
Coralliophila brevis (Blainville, 1832)	10?0100?11??1100010???10?
Coralliophila meyendorffii (Calcara, 1845)	1000100111001100010 10?100
Leptoconchus cumingii Deshayes, 1863	10??0000?201?00001??00010
Leptoconchus peronii (Lamarck, 1818)	10??0000?201?00001??00010
Leptoconchus lamarckii (Deshayes, 1863)	10??0000?201?00001??00000
Coralliophila squamosa (Bivona, 1838)	?1?1100??100100001?????0?
Paziella pazi (Crosse, 1869)	0011100?0000100000 1021101

Appendix 3

Character state changes

Changes in characters are based on the strict consensus tree of Figure 1. The node numbers correspond to those given in the same figure. Arrows indicate the direction of change. Synapomorphies are indicated with bold letters and potential synapomorphies with * (for explanation see text).

node 1 → node 2: **character 17**: 0 → 1.
node 1 → node 3: character 7: 1/2 → 2, **character 20**: 0 → 2, **character 21**: 0 → 1
node 2 → node 4: **character 0**: 0/1 → 1, character 1: 1 → 0, character 2*: 1 → 0, character 3: 1 → 0, character 8*: 0/1 → 0, character 18*: 0/1 → 0, character 19*: 0/1 → 0.
node 3 → node 5: **character 12**: 1 → 0.
node 3 → node 6: character 1: 1 → 0, character 9: 1 → 0/1.
node 4 → node 7: character 13: 0 → 1, character 22: 0/1 → 1.
node 4 → node 8: character 4: 1 → 0, character 7: 1 → 0, character 9: 1 → 2, character 11: 0 → 1, character 22: 0/1 → 0.
node 6 → node 10: character 9: 0/1 → 0, character 11: 0 → 1.
node 6 → node 9: character 3: 1 → 0, character 7: 2 → 0, character 13: 0 → 1.
node 8 → node 11: **character 23**: 0 → 1.
node 10 → node 12: **character 6**: 0 → 1.

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On the morphology and taxonomic position of *Babylonia* (Neogastropoda: Babyloniidae)

M. Gerry Harasewych & Yuri I. Kantor

KEY WORDS: *Babylonia*, Babyloniidae, taxonomic position, anatomy, phylogenetic analysis, cytochrome *c* oxidase I sequence.

ABSTRACT *Babylonia* Schlüter, 1838 is a conchologically distinctive and commercially important genus of neogastropods traditionally assigned to the family Buccinidae. External morphology and anatomy of several species of *Babylonia* have been studied in detail for the first time. All studied the species of this genus possess an unpaired accessory salivary gland, and two of six studied species (*B. areolata* and *B. japonica*) have a rectal gland. The radula of all species is distinctive and differs markedly from that of any buccinoidean in having cusps along the outer edges of the rachidian teeth, and in the morphology of the three strongly buttressed central cusps, which emanate from the anterior edge of the basal plate. Cladistic analyses of anatomical data as well as of partial DNA sequences of the cytochrome *c* oxidase I gene revealed that the genus *Babylonia* is not closely related to Buccinoidea, but has close affinities to the volutoidean families Volutidae and Olividae. More detailed comparisons that include a broader representation of volutoidean families will be required to more precisely determine the sister group relationship of the family Babyloniidae Kuroda, Habe and Oyama, 1971.

RIASSUNTO *Babylonia* Schlüter, 1838 è un genere di neogasteropodi ben caratterizzato conchigliologicamente e di una certa importanza commerciale, tradizionalmente assegnata alla famiglia Buccinidae. In questo lavoro si presentano dati derivati dallo studio della morfologia esterna e dell'anatomia di alcune specie, per la prima volta ad un elevato livello di dettaglio. Tutte le specie studiate posseggono una ghiandola salivare accessoria impari, e due delle sei specie studiate (*B. areolata* e *B. japonica*) hanno una ghiandola rettale. La radula di tutte le specie è nettamente distinta e differisce rimarchevolmente da quella di qualunque buccinoideo nell'avere cuspidi lungo il bordo esterno del dente rachidiano, e nella morfologia delle tre cuspidi centrali, che originano dal bordo anteriore della lamina basale.
Un'analisi cladistica dei caratteri anatomici insieme ai dati da sequenze parziali del gene per la citocromo *c* ossidasi I (COI) mostrano come il genere *Babylonia* non sia strettamente correlato ai Buccinoidea, ma abbia forti affinità con i Volutoidea, in particolare con le famiglie Volutidae ed Olividae. Comparazioni più dettagliate che includano una più ampia rappresentanza delle famiglie volutoidee saranno necessarie per determinare più precisamente le relazioni di sister-group della famiglia Babyloniidae Kuroda, Habe & Oyama, 1971.

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INTRODUCTION

The genus *Babylonia* Schlüter, 1838, encompasses a group of conchologically distinctive and commercially important neogastropods that inhabit soft bottoms at littoral and sublittoral depths along the western and northern margins of the Indian Ocean, the Indonesian Archipelago, the Philippines, and Japan. While the species level systematics of *Babylonia* is stable and well documented (e.g., HABE, 1965; ALTENA & GITTENBERGER, 1981), the taxonomic rank and relationships of this group within Neogastropoda have a long and convoluted history.

This genus was well known to nineteenth century authors as *Eburna*, originally proposed by LAMARCK (1801:78) to include a single southern Caribbean species, *Buccinum glabratum* Linné, 1758, now referred to the subfamily Ancillinae of the family Olividae. Later, LAMARCK (1822) expanded the genus to contain four additional species today included in *Babylonia*. Lamarck placed *Eburna* within the family Purpurifera, and suggested an affinity to *Buccinum*, noting (LAMARCK, 1822:280) that *Eburna* is "Distinguished from *Buccinum* by the singular position of the umbilicus, of the columella, which is also produced so as to form a canal, which occupies the rest of the left lip" (translation cited from GOULD, 1833). Recognizing that LAMARCK's 1822 formulation of *Eburna* differed markedly from his original usage of the genus, SCHLÜTER, (1838:18) proposed the genus *Babylonia*, without diagnosis or discussion, including only *Buccinum spirata*. Presumably unaware of SCHLÜTER's 1838 work, GRAY (1847) introduced name *Latrunculus*, also without description or discussion, listing *Eburna*, sp. *Lam.* 1822, not 1801, in its synonymy. He included this genus within Buccinidae (as Buccinina).

The majority of 19th century iconographies include a monograph of the genus *Eburna* (e.g., KIENER 1835; REEVE, 1849; SOWERBY, 1859; TRYON, 1881). SOWERBY (1859) noted that "Authors have generally agreed to remove from this genus the [type species] *Buccinum glabratum* of Linneus, which is an *Ancillaria*, ... the remaining species form a very compact and well-defined genus." TRYON (1881) recognized that, based on its type species, "*Eburna* must become a synonym of *Ancillaria*" but continued to use *Eburna*, noting that "Naturalists have done much to render science and themselves contemptible by expending their time upon the nomenclature, instead of the structure and habits of the animals." With the increasing codification of nomenclatural protocols toward the end of the 19th century (see MELVILLE, 1995), *Babylonia* gained widespread usage for this group early in the 20th century (e.g., THIELE, 1929, WENZ, 1943).

Most authors have attributed *Babylonia* (some using one of its various synonyms – *Eburna*, *Latrunculus*, *Peridipsaccus*) to the family Buccinidae (e.g., GRAY, 1847; H. & A. ADAMS, 1853) based primarily on shell morphology. The gross external morphology of the animal and the radula of several species were described or figured by a number of authors (e.g., KIENER, 1835; ADAMS & REEVE, 1848; EYDOUX & SOULEYET, 1852; SOWERBY, 1902; ALTENA & GITTENBERGER, 1983; RIEDEL, 2000). REEVE (1849) commented that the animal of *Babylonia* is so similar to that of *Buccinum* that the species should hardly be separated, were it not

for differences in shell morphology. Indeed, the distinctive morphology of the shell prompted several authors to erect supra-generic taxa (Eburninae SWAINSON, 1840, as a subfamily of Turbinellidae; Babyloniinae KURODA, HABE & OYAMA, 1971, as a subfamily of Buccinidae; Babyloniidae GORYACHEV, 1987, as a family of Buccinoidea) to **encompass this group.**

Over the past year, we were able to examine living and preserved specimens of several species of *Babylonia*. Dissections supplemented with histological studies revealed that the anatomical organization of all species of *Babylonia* available to us differs significantly from that of all known buccinoideans, and is incompatible with the inclusion of *Babylonia* within the Buccinoidea. In this paper, we document the anatomical features of six of the 13 Recent species, and present the results of cladistic analyses based on this morphological data in order to discern the phylogenetic relationships of *Babylonia*. These findings are corroborated by phylogenetic analyses of partial sequences of the cytochrome *c* oxidase I gene of *Babylonia japonica* and representative neogastropod taxa.

MATERIALS AND METHODS

Specimens of *Babylonia areolata* and *B. spirata* were collected and dissected living. Shells of living *Babylonia japonica* were cracked in a vise and the animals immersed and shipped in 85% ethanol. Samples of the remaining species were received in varying states of preservation.

Ciliary currents in the stomach were traced by applying carmine particles finely dispersed in seawater while dissecting living specimens. When warranted, tissues were embedded in paraffin, sectioned at 10 µm and stained using Masson Triple Stain. Protoconch, shell ultrastructure and radulae were coated with carbon and gold and examined using a Leica Stereoscan 440 Scanning Electron Microscope.

Taxa, characters, and the data matrix used for phylogenetic analyses of the relationships of *Babylonia* based on morphological characters are itemized in Table 1, which also references the sources of data for selected neogastropod taxa used in the analysis. *Splendrillia chathamensis* Sysoev & Kantor, 1989, a primitive member of the Conoidea (TAYLOR, KANTOR & SYSOEV, 1993), the sister taxon or Rachiglossa (TAYLOR & MORRIS, 1988; KANTOR, 1996), was selected to serve as the outgroup for this analysis. Representative taxa from major lineages within Buccinoidea as well as other rachiglossan groups were selected based on availability of published data.

The six species of *Babylonia* that we have studied have identical character state distributions except for the presence or absence of a discernible rectal gland. The data matrix includes *Babylonia areolata* as a representative of species that have a rectal gland (*B. areolata*, *B. japonica*), and *Babylonia spirata* as a representative of species that lack a rectal gland (*B. spirata*, *B. papillaris*, *B. lutosa*). We were not able to determine if *B. zeylanica* has a rectal gland.

DNA was extracted from an ethanol preserved specimen of *Babylonia japonica*. Protocols for DNA extraction, PCR amplification, DNA sequencing, are identical to those reported by HARASEWYCH ET AL. (1997a). Primers for PCR amplification and sequencing of the cytochrome c oxidase I gene fragment are from FOLMER ET AL. (1994). PCR product was purified using a Wizard PCR Purification Kit (Promega) and sequenced on an Applied Biosystems 377 Fluorescent Sequencer using Prism Sequencing Kits according to the manufacturer's protocols. The partial CO I sequence of *Babylonia japonica* and four buccinoidean taxa were aligned against previously published neogastropod CO I sequences from taxa selected to represent as closely as practical the higher taxa contained in the morphological data set. Table 2 lists the taxa used in this study, their source, voucher specimen information, and GenBank sequence accession numbers. The sequences were aligned using Clustal W (THOMPSON ET AL., 1994) with minor manual adjustments.

Maximum parsimony analyses of the morphological and molecular data were performed using PAUP 4.02 (SWOFFORD, 1998).

Abbreviations for the museums:

NM – Natal Museum, Pitermaritzburg, South Africa;

USNM – National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.

ZMMU – Zoological Museum of Moscow State University, Moscow, Russia.

RESULTS

Anatomical Data

Family: BABYLONIIDAE Kuroda, Habe & Oyama, 1971

Synonymy – Eburninae Swainson, 1840:305.
Babyloniinae Kuroda, Habe & Oyama, 1971:*250*.
Babyloniidae Goryachev, 1987: 35.

Remarks: As noted in the introduction, several suprageneric taxa have been proposed for *Babylonia*. The oldest name is Eburninae, which was proposed by SWAINSON (1840) as a subfamily of Turbinellidae. Swainson's generic concept of *Eburna* was identical to the current concept of *Babylonia*, as he included as an example two species, *E. spirata* and *E. pacifica* [= *B. lutosa* (Lamarck, 1822)]. Nevertheless, the name is invalid, since it is based on the genus *Eburna* Lamarck, 1801, which is a valid genus of Olividae.

More recently, KURODA, HABE AND OYAMA (1971) established the subfamily Babyloniinae within Buccinidae. As their diagnosis was provided in Japanese only, we include the English translation here (translated by Paul Callomon): "Shells of medium size, strong and robust; oviform to oval. Spire conical, whorls mildly convex, sutures shallow or forming a groove. Shell surface smooth, with pattern of spots, covered with thick periostracum. Body whorl large, fasciole prominent, umbilicus open or enclosed by extension of apertural margin. Aperture oviform, outer lip curved, inner margin smooth; siphonal canal short, broad and open". No anatomical characters were included in the diagnosis.

GORYACHEV (1987), apparently unaware of KURODA, HABE AND OYAMA's (1971) taxon, proposed the family Babyloniidae within Buccinoidea. As Goryachev's taxon was proposed without diagnosis and description, it should be considered invalid (ICZN Article 13.2). The oldest available name is that of KURODA, HABE AND OYAMA (1971), who should be considered the authors of the family Babyloniidae. This family includes a single genus, *Babylonia* Schlüter, 1838

Genus: *Babylonia* Schlüter, 1838

Synonymy - *Eburna* Lamarck, 1822:281 (not Lamarck, 1801).
Babylonia Schlüter, 1838:18. [type species, by monotypy, *Buccinum spiratum* Linnaeus, 1758]. *Latrunculus* Gray, 1847: 139. [type species, by subsequent designation (ALTENA & GITTENBERGER, 1981:10), *Babylonia spirata* (Linnaeus, 1758)]. *Peridipsaccus* Rovereto, 1900: 168. [type species, by subsequent designation (ALTENA & GITTENBERGER, 1981:10), *Babylonia spirata valentiana* (Swainson, 1822)].
Zemiropsis Thiele, 1929:332. [type species, by monotypy, *Eburna papillaris* Sowerby, 1825].

Remarks: ROVERETO (1900) considered *Latrunculus* Gray, 1847 to be the valid generic name for *Babylonia*, and placed *Eburna* Lamarck, 1822 (non 1801) and *Babylonia* Schlüter, 1838, which he considered to be a *nomen nudus* (sic!), in its synonymy. He also introduced the section (=subgenus) *Peridipsaccus* [type species, "*L. mollianus* Chemnitz" = *Babylonia spirata valentiana* (Swainson, 1822)] for the non-umbilicate species, and included *Eburna papillaris* Sowerby, 1825. THIELE (1929) later proposed the monotypic genus *Zemiropsis* [type species, *Eburna papillaris* Sowerby, 1825] and placed it in the subfamily Pseudolivinae of the family Olividae, noting that "*the systematic position of this species, which was previously placed in Babylonia, is uncertain without knowledge of the animal*" [THIELE, 1992:505]. ALTENA AND GITTENBERGER (1981) distinguished *Zemiropsis* from *Babylonia* on the basis of conchological differences as well as on the presence of a pronounced medial pedal tentacle in *Zemiropsis*, and its absence in *Babylonia*.

All species of *Babylonia* that we were able to examine had a medial pedal tentacle. While this tentacle tends to be strongly contracted and weakly discernible in preserved material, it is prominent in living specimens of *Babylonia areolata, B. spirata* and *B. zeylanica*. As *B. papillaris* does not differ anatomically from other species of *Babylonia*, we see little justification for retaining the name *Zemiropsis*, which becomes a synonym of *Peridipsaccus* and *Babylonia.*

***Babylonia (Babylonia) areolata* (Link, 1807)**
Figures 1A, 2A-H, 3A-D, 4A-D, 5A-D

Material examined: ZMMU Lc-25174 (voucher material — sections and radular preparations), Nha Trang, Central Vietnam, coll. Yu. Kantor, November 1999. 3 ♀ specimens.

External anatomy: (Fig. 2A). Soft tissues comprise approximately 3½ whorls. Mantle cavity spans ~ ⅓ whorl. Mantle edge thick, does not cover head. Columellar muscle short, broad, spanning ~ ½ whorl. Nephridium (Fig. 2A, nep) narrow, covering ~ ¼ whorl. Foot moderately large, elongate, oval (L/W ≈ 1.75), terminating posteriorly in small pedal tentacle (Fig. 2A, ped.t). Base color of preserved specimen yellowish, with dorsal surfaces of head, tentacles, siphon, posterior part of foot mottled with dark grayish black. Head with conical, tapering tentacles with black eyes at their bases. Siphon short, muscular. Operculum leaf-shaped, brownish, with terminal nucleus.

Mantle cavity: (Fig. 2B). Mantle thick, opaque, with smooth, thickened edge that is outwardly reflected in preserved specimens. Mantle cavity slightly longer than wide. Siphon (Figs. 2A-B, s) short, muscular, thick-walled, extending substantially beyond mantle edge. Osphradium (Figs. 2A-B, os) small, bipectinate, slightly asymmetrical (ventral lamellae slightly larger than dorsal lamellae), with narrow axis, spanning ~ 0.4 mantle cavity length, < ½ of ctenidium length. Ctenidium (Figs. 2A-B, ct) large, narrow, spanning > ¾ mantle cavity length, more than twice as wide as osphradium. Ctenidial lamellae high, subtriangular posteriorly, become gradually lower anteriorly, with an overhanging extension along their dorsal crests. Hypobranchial gland (Fig. 2B, hg) of three large, thickened, oblique folds, covered by layer of mucus. Rectum (Fig. 2B, re) broad, thick-walled along its entire length, embedded in the capsule gland (Fig. 2, cg), free at terminal end. Rectal gland, short, narrow, tubular, barely visible through mantle wall near anterior margin of rectum. Gland lined with epithelium of low cells with few melanin granules (Fig. 4A-C, rg). Gland opens into mantle cavity by narrow duct posterior to anus. Anal opening (Fig. 2B, a) laterally compressed slit. Capsule gland broad, laterally compressed. Bursa copulatrix large and swollen.

Alimentary system: (Figs. 2C-H, 3A-D, 5A-D). Mouth opening (Fig. 2G) in form of vertical slit, unlike mouth of Buccinidae, which is triangular. Retracted proboscis 13 mm long, extended proboscis 26 mm long, with proboscis sheath and rhychodeum protracted to form proboscis wall. Proboscis wall ~1.2 mm thick at its distal most margin, becoming abruptly thinner (~0.4 mm) posterior to buccal cavity, gradually decreasing in thickness, reaching ~0.2 mm in thickness at the posteriormost limit of the extended proboscis. Proboscis retractor muscles (Figs. 2C-D, prr) thin, numerous, equal in size, attached to inner proboscis wall in a ring at about ⅕ the distance from the proximal to the distal end.

Oesophagus (Fig. 2H, oe) narrow, flattened dorso-ventrally, constricts before passing through nerve ring (Fig. 2D-E, ao), expands posterior to nerve ring (Figs. 2C-E, poe). Epithelium in this expanded oesophagus yellow-orange, producing large amount of orange mucus. Folds on dorsal wall of oesophagus enlarged, seemingly glandular. Posterior region of oesophagus narrow, light yellow, with dorsal folds less prominent, of the same thickness as on the other walls of the oesophagus. The valve of Leiblein and gland of Leiblein absent. Expanded region of oesophagus similar to the *glande framboisée* of Muricidae (e.g. FRETTER & GRAHAM, 1994, fig. 116 — *Nucella lapillus*), but less pronounced.

Salivary glands (Figs. 2C-D, F, sg) very small, fused, with indistinct border, cover most of dorsal surface of nerve ring. Salivary ducts run loosely along both sides of anterior oesophagus, entering into its wall just dorsal to opening of radular diverticulum (Fig. 2H, sd). Accessory salivary gland, single, narrow, tubu-

lar, slightly shorter than retracted proboscis. Duct of accessory salivary gland runs medially under esophagus, entering buccal cavity anterior to radular diverticulum. Salivary glands, nerve ring, posterior esophagus enveloped in dense connective tissue. Buccal mass longer than retracted proboscis, extending beyond its posterior margin. Radular diverticulum (Fig. 2H, rd) very long, equal in length to retracted proboscis. Odontophore equal in length to buccal mass, composed of paired subradular cartilages fused anteriorly. Radula triserial, 9.1 mm long, 1.18 mm wide (0.031 SL), composed of 40 rows of teeth, of which 3 are nascent. Rachidian teeth with 5 cusps. Three cusps large, closely spaced, equal in length, radiating from midpoint of tooth, one shorter, broader, triangular cusp at each lateral end of tooth. Rachidian teeth attached to radular membrane by very short base, with central cusp buttressed posteriorly (Fig. 5D). Central cusp with deep indentation on its anterior dorsal surface, accommodating central cusp tip of anteriorly adjacent tooth (Fig. 5C-D). Lateral teeth bicuspid, with outer cusp >2 times longer than inner cusp.

Table 1. Data matrix and descriptions of morphological characters and character states used in cladistic analyses. Missing characters are indicated by "?".

	Characters			
	1 0	2 0	2 4	
Taxa				Sources of anatomical data:
Splendrillia chathamensis	0000200021	013020000?	?011	Sysoev & Kantor, 1989
Babylonia areolata	1111120110	1130001101	2110	Herein
Babylonia spirata	1111120110	1130001101	2210	Herein
Leucozonia nassa	0101220121	0000200001	0211	Marcus & Marcus, 1962a + unpublished observations
Melongena melongena	0101220111	0010200001	2212	Unpublished observations
Columbella mercatoria	0101220132	0001200011	0210	Marcus & Marcus, 1962b
Ilyanassa obsoleta	1101220121	0010200211	121?	Brown, 1969
Buccinum undatum	0101220121	0000210200	0212	Dakin, 1912 + unpublished observations
Chlanidota densesculpta	0101220121	0001200001	0212	Harasewych & Kantor, 1999
Neptunea antiqua	0101220121	0010200200	0212	Goryachev & Kantor, 1983 + Smith, 1967
Latiromitra cryptodon	0000110100	00100001??	0001	Bouchet & Kantor, 2000
Oliva oliva	0101111100	0010000200	2010	Kantor & Tursch, 1998
Vasum muricatum	0101210111	0000200101	0000	Mediskaya et al. 1996
Vexillum luculentum	0001010101	0000100101	1010	Ponder, 1972
Alcithoe arabica	0101011240	0020000001	2010	Ponder, 1970
Xymenopsis muriciformis	0101010101	0000000201	1011	Pastorino & Harasewych, 2000

Morphological Characters and Character States:

1. Posterior pedal tentacle: (0) Absent; (1) Present.
2. Buccal mass: (0) at base of proboscis; (1) at distal end of proboscis.
3. Radular diverticulum: (0) < ⅓ of retracted proboscis length; (1) > ⅓ of retracted proboscis length.
4. Radular retractor muscles: (0) passing through nerve ring, attached to the columellar muscle; (1) not passing through the nerve ring, attached to the proboscis walls.
5. Accessory salivary glands: (0) paired; (2) single, not embedded in primary salivary gland; (3) absent.
6. Ducts of the primary salivary glands: (0) free, entering the walls of the buccal cavity; (1) entering the walls of oesophagus just anterior to valve of Leiblein, embedded in the walls of the oesophagus along; (2) free along most of the length, entering oesophagus walls posterior to buccal cavity.
7. Primary salivary glands: (0) Acinous; (1) Ramified tubular.
8. Number of radular teeth in a transverse row: (0) 5; (1) 3; (2) 1.
9. Lateral teeth: (0) Unicuspid; (1) Bicuspid; (2) 3 or more cusps; (3) Multicuspid with narrow base; (4) absent.
10. Cusps of the rachidian teeth: (0) Emanating from the anterior edge of the basal plate; (1) Emanating from mid-portion or posterior edge of the basal plate; (2) Absent.
11. Cusp at lateral edge of rachidian: (0) Absent; (1) Present.
12. Valve of Leiblein: (0) Present; (1) Absent.
13. Gland of Leiblein: (0) Large; (1) Reduced; (2) Tubular, convoluted; (3) Absent.
14. Posterior esophagus: (0) Not glandular; (1) Glandular.
15. Dorsal glandular folds of the oesophagus: (0) Present; (1) Present, stripped from the oesophagus; (2) Absent.
16. Oeosphageal caecum: (0) Absent; (1) Present.
17. Stomach: (0) Not covered by nephridium; (1) Covered by nephridium.
18. Posterior mixing area (caecum): (0)Absent; (1) Present, small; (2) Present, large.
19. Gastric shield: (0) Absent; (1) Present.
20. Posterior sorting area in stomach: (0) Present; (1) Absent.
21. Ducts of the digestive system: (0) Paired, broadly separated; (1) Paired, closely spaced; (2) Fused into single duct prior to entering stomach.
22. Rectal gland: (0) Present, opening into rectum; (1) Present, opening into mantle cavity; (2) Absent.
23. Seminal duct: (0) Open; (1) Closed.
24. Penis with terminal papilla: (0) Absent; (1) Present, simple, tubular; (2) Present, surrounded by circular fold at its base.

Stomach very small (Fig. 3), simple, U-shaped, with very short caecum (= posterior mixing area) (Fig. 3A,C, pma), mostly or completely covered by nephridium (Fig. 3D). Transition of posterior oesophagus into stomach marked by changes in epithelium color, presence of the powerful sphincter (Fig. 3C, sph). Walls of caecum much thicker, more muscular that remaining regions of stomach, dark pink in living animals, suggesting that caecum is capable of contracting and mechanically processing food. Epithelial folds of caecum mostly transverse. Single, thick digestive gland duct (Fig. 3A, ddg) opens into stomach in deep pouch (Fig. 3C, dp), giving rise to pronounced, raised fold (Fig. 3C, lf) along floor of stomach that becomes progressively thicker in posterior part of stomach, terminating abruptly at transition to intestine. Several smaller longitudinal folds are present along stomach floor, but no separation into dorsal and ventral channels is evident in stomach. Examination of ciliary currents in living animals revealed strongest currents to run along longitudinal fold (Fig. 3C, arrows; arrow size corresponds to current strength), with slight, turbulent ciliary currents in area of duct pouch, and slight flow of particles out of digestive gland duct into stomach. Duct of digestive gland bifurcates at some distance from stomach, each branch leading to lobe of digestive gland. Lobes of digestive gland fused, without clear demarcation.

Babylonia papillaris (Sowerby, 1825)
Figures 1B, 5E-H, 6A-I

Material examined: NM V7705. Jeffrey's Bay, Cape of Good Hope, South Africa, dredged in 70 m. col. W. Immelman, January 2000, 1 shell + 1 animal without shell, now in ZMMU Lc-25250.

External anatomy: (Fig. 6A). Soft tissues comprise approximately 3 whorls. Mantle cavity spans ~ ½ whorl. Mantle edge thin, does not cover head. Nephridium wide, covering ~ ⅓ whorl (Fig. 6B, nep). Foot moderately large, elongate, oval (L/W ≈ 1.5), terminating posteriorly in distinct pedal tentacle (Fig. 6A, ped.t). Base color of preserved specimen light cream, with dorsal-lateral surfaces of foot, tentacles, siphon mottled with pale red-orange. Head with long conical, tapering tentacles (Fig. 6A, cep.t) with large black eyes at their bases. Operculum leaf-shaped, yellow, transparent, with terminal nucleus.

Mantle cavity: Mantle thin, translucent. Mantle edge slightly thickened, smooth. Length of mantle cavity equals width. Siphon long, muscular, thick-walled, extending substantially beyond mantle edge, slightly pigmented dorsally with pale orange spots. Osphradium small bipectinate, slightly asymmet-

Table 2. Taxa, collection localities, voucher material and GenBank accession numbers for Cytochrome *c* oxidase I sequences used in this study.

CONOIDEA		
Conus jaspideus Gabb, 1868	*ex* Harasewych *et al.* 1997b	U86337
Hastula cinerea (Born, 1778)	*ex* Harasewych *et al.* 1997b	U86336
BUCCINOIDEA		
Fasciolaria tulipa (Linné, 1785)	Berry Islands, Bahamas USNM 894804	AF373884
Pleuroploca gigantea (Kiener, 1840)	Ft. Pierce, Florida USNM 894805	AF373885
Buccinum oedematum Dall, 1907	*ex* Harasewych *et al.* 1997b	U86327
Neptunea antiqua (Linné, 1758)	Millport, Scotland USNM 894806	AF373886
Neptunea polycostata Scarlatto, 1952	*ex* Harasewych *et al.* 1997b	U86326
Busycotypus canaliculatus (Linné, 1758)	*ex* Harasewych *et al.* 1997b	U86325
Busycon carica (Gmelin, 1791)	*ex* Harasewych *et al.* 1997b	U86323
Busycon sinistrum Hollister, 1958	*ex* Harasewych *et al.* 1997b	U86324
Busycon perversum (Linné, 1758)	Celestun, Mexico USNM 894807	AF373887
"VOLUTOIDEA"		
Turbinella angulata (Lightfoot, 1786)	*ex* Harasewych *et al.* 1997b	U86332
Oliva sayana Ravenel, 1834	*ex* Harasewych *et al.* 1997b	U86333
Babylonia japonica (Reeve, 1842)	Osaka, Japan USNM 894808	AF373888
Arctomelon stearnsii (Dall, 1872)	*ex* Harasewych *et al.* 1997b	U86334
Scaphella junonia (Lamarck, 1804)	*ex* Harasewych *et al.* 1997b	U86335
MURICOIDEA		
Coralliophila abbreviata (Lamarck, 1816)	*ex* Harasewych *et al.* 1997b	U86331
Thais haemastoma canaliculata (Gray, 1839)	*ex* Harasewych *et al.* 1997b	U86330
Phyllonotus pomum (Gmelin, 1791)	*ex* Harasewych *et al.* 1997b	U86328
*Murex troscheli*Lischke, 1868	*ex* Harasewych *et al.* 1997b	U86329

rical (dorsal lamellae slightly wider than ventral lamellae), with very narrow axis, spanning ~ 0.45 mantle cavity length, slightly < ½ ctenidium length. Ctenidium (Fig. 6B, ct) large, very broad, spanning > 0.8 mantle cavity length, three times as wide as osphradium. Ctenidial lamellae low, subtriangular, similar in shape throughout ctenidium length. Hypobranchial gland weakly differentiated, lacking distinct folds. Rectum broad, thin-walled, transparent, free at its terminal end. Anal opening round, with slightly thickened reflected edge. Capsule gland broad, rounded in transverse section (Fig. 6B, cg). Bursa copulatrix large, swollen. Ingesting gland (Fig. 6B, ig) at posterior portion of capsule gland very large, dark. Rectal gland absent.

Alimentary system: (Figs. 5C-I). Mouth opening in form of triangular slit. Partially extended proboscis 16 mm long, with slightly folded walls. Proboscis wall ~1.6 mm thick at distalmost margin (Fig. 6G), becoming thinner (~ 0.25 mm) posterior to buccal cavity, sharply decreasing in thickness in middle part and gradually thickening in the posteriormost limit, reaching ~0.4 mm. Proboscis retractors (Figs. 5C-E, prr) numerous, arranged in two bundles, attached to mid-lateral sides of the rhynchodaeum (Figs. 5D-E, prr. Anterior oesophagus narrow, flattened dorso-ventrally, constricted before passing through nerve ring, sharply expanded posterior to ring. Epithelium in expanded region slightly darker than in rest of oesophagus.
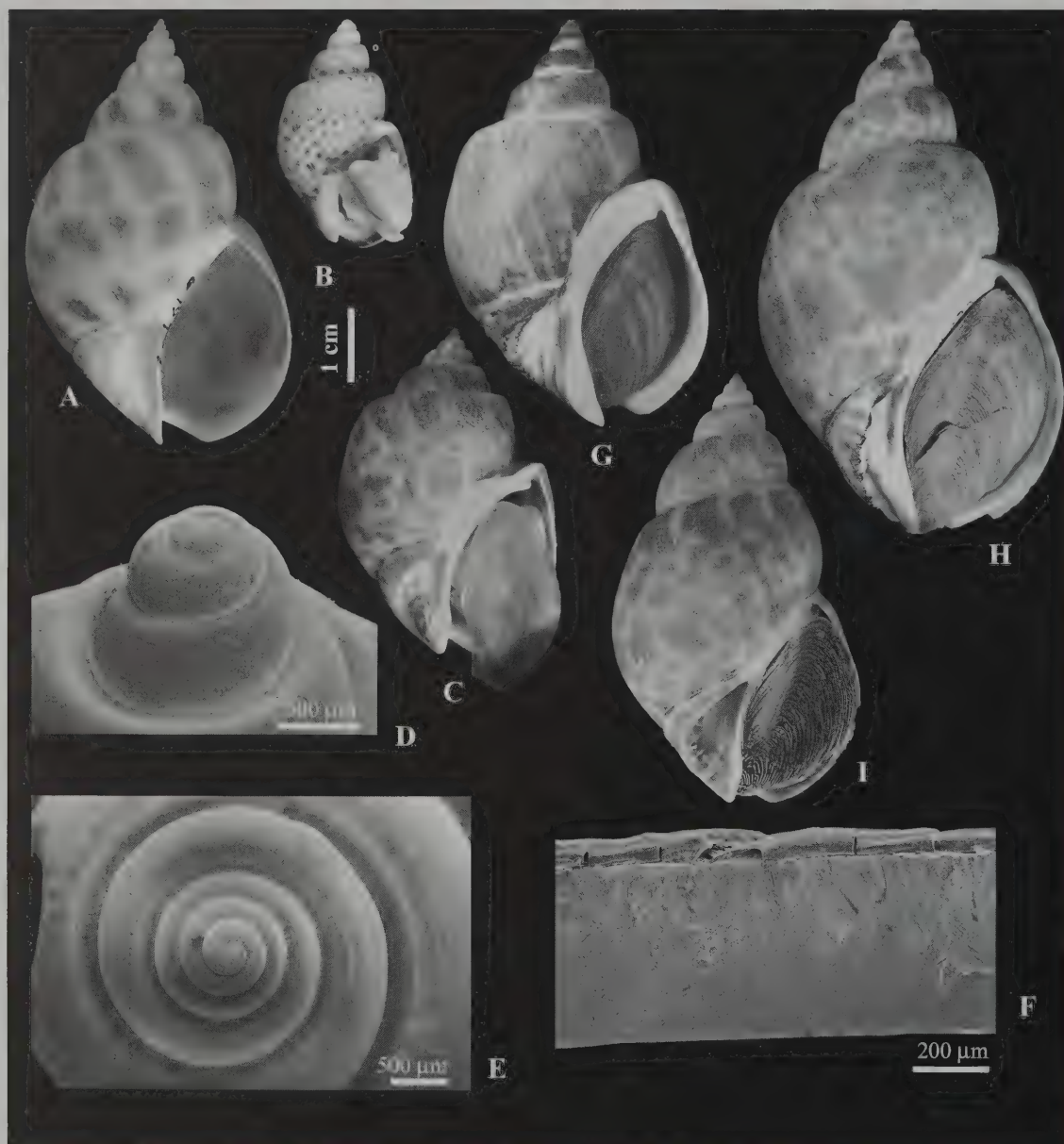
Figure 1. Shells of the species of *Babylonia* examined in this study. A — *Babylonia areolata* (Link, 1807), Hua Him, Thailand, USNM 679439. B — *B. papillaris* (Sowerby, 1825), South Africa, Cape of Good Hope, Jeffrey's Bay, NM V7705 (now in ZMMU). C — *B. spirata* (Linnaeus, 1758), South India, off Tuticorin. **D-E** — lateral and apical view of protoconch of juvenile of *B. spirata*, USNM 443286, Back Bay, Bombay. F — shell ultrastructure of *B. spirata* (middle portion of outer lip), South India, off Rameswaram. G — *B. lutosa* (Lamarck, 1822), Borneo, USNM 31342. H — *B. japonica* (Reeve, 1842), Hashimoto, Japan, USNM 665016. I — *B. zeylanica* (Bruguière, 1789) South India, off Tuticorin, ZMMU Lc-25173.
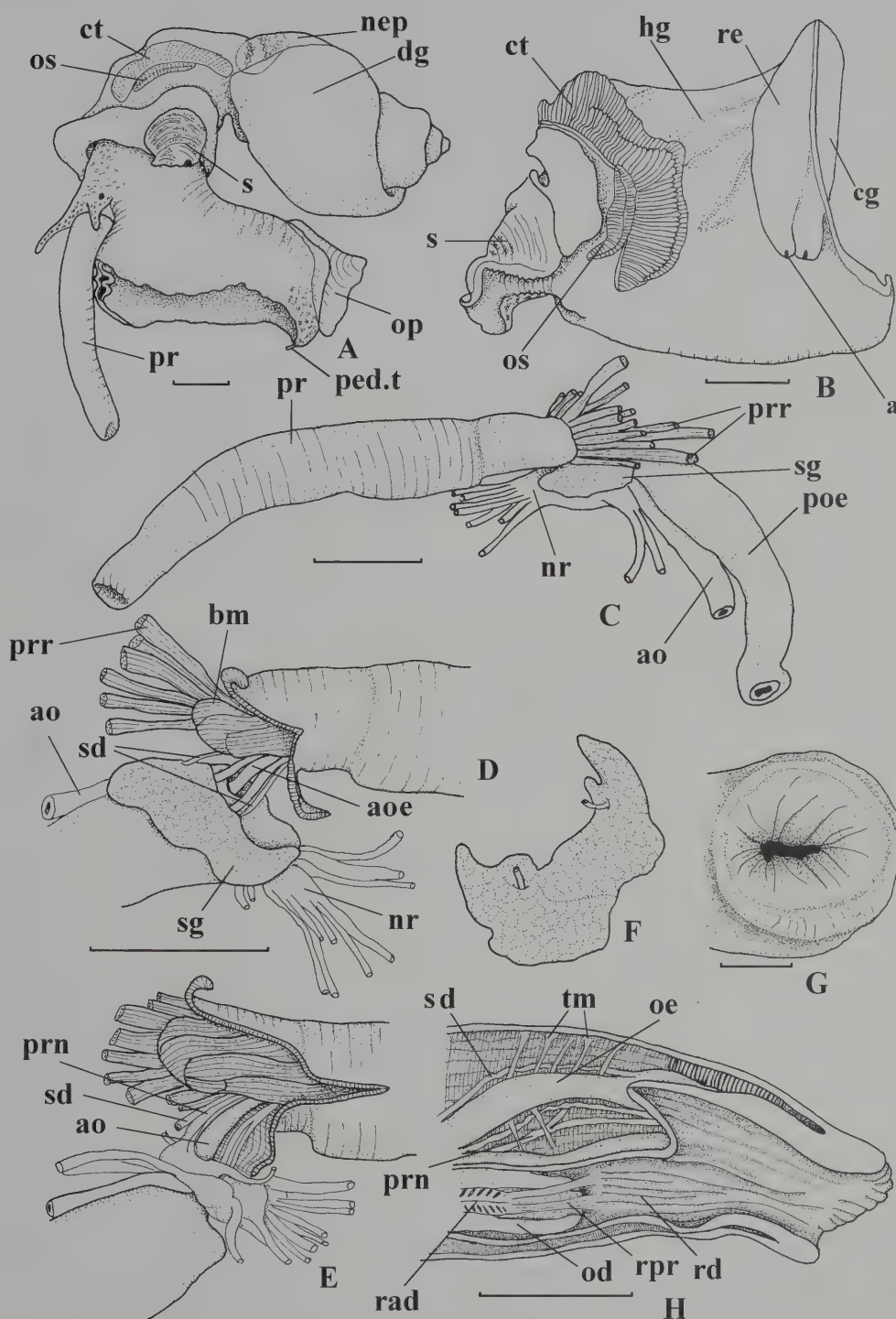
Figure 2. Anatomy of *Babylonia areolata* (Link, 1807). A — Lateral view of animal removed from the shell. B — Mantle cavity organs of ♀ specimen. C — Left lateral view of anterior alimentary system. D — Right lateral view of the posterior part of the proboscis (oesophagus turned clockwise to show salivary gland). E — Right lateral view of the posterior part of the proboscis (salivary gland removed to show the circumoesophageal nerve ring, oesophagus turned clockwise, proboscis opened laterally). F — Dorsal view of the salivary gland. G — Anterior view of the proboscis tip. H — Anterior part of the proboscis opened laterally from the right side. Scale bars = 5 mm for A-F, H; 1 mm for G.

Abbreviations: ao, anterior aorta; bm, buccal mass; cg, capsule gland; ct, ctenidium; dg, digestive gland; hg, hypobranchial gland; nep, nephridium; nr, circumoesophageal nerve ring; od, odontophore; oe, oesophagus; op, operculum; os, osphradium; ped.t, pedal tentacle; poe, posterior oesophagus; pr, proboscis; prr, proboscis retractor muscles; rad, radula; rd, radular diverticulum; re, rectum; rpr, radular protractors; s, siphon; sd, salivary duct; sg, salivary gland; tm, tensor muscles.

Folds on dorsal wall of oesophagus enlarged, seemingly glandular (Fig. 6H, dgf). Posterior region of oesophagus narrow, with dorsal folds of same size as folds on ventral, lateral walls. Valve of Leiblein and gland of Leiblein absent.

Salivary glands (Fig. 6C-F, sg) dorsal to nerve ring, medium-sized, fused, with indistinct border. Left salivary gland extends further posteriorly than right. Salivary ducts (Fig. 6G, sd) coiled, running loosely along both sides of anterior oesophagus, entering into its wall slightly posterior to opening of radular diverticulum. Accessory salivary gland (Fig. 6G, asg) single, narrow, tubular, slightly longer than retracted proboscis. Duct of accessory salivary gland runs medially under esophagus, entering buccal cavity anterior to radular diverticulum. Salivary glands, nerve ring, posterior esophagus enveloped in dense connective tissue. Buccal mass longer than retracted proboscis, extending beyond its posterior margin (Figs. 6C-E, G, bm). Radular diverticulum (Fig. 6G, rd) ~½ of retracted proboscis. Odontophore ~⅔ of buccal mass length, composed of paired subradular cartilages fused anteriorly. Radula triserial, 9.45 mm long, 1.27 mm wide, composed of 46 rows of teeth (Fig. 5E-H). Rachidian teeth with 3 large, stout, closely spaced, nearly parallel cusps concentrated at middle of tooth, central cusp slightly longer, much broader than flanking cusps. Single, shorter cusps present near lateral margins of rachidian tooth. Each of 3 median cusps with deep indentations on anterior, dorsal surfaces, accommodating tips of cusps of anteriorly adjacent tooth (Fig. 5G-F). Lateral teeth bicuspid, with outer cusp >2 times longer than inner cusp.

Stomach very small (Fig. 6B, I st), simple, U-shaped, with very short caecum, mostly covered by nephridium. Transition of posterior oesophagus into stomach marked by changes in epithelium, presence of powerful sphincter (Fig. 6I, sph). Epithelial folds of caecum (Fig. 6I, pma) positioned at obtuse angle to folds of posterior oesophagus. Single thick duct of digestive system opens into deep pouch (Fig. 6I, dp). Two prominent folds run along floor of stomach from deep pouch to intestine (Fig. 6I, lf). Rest of stomach lined with weak longitudinal folds. Stomach not separated into dorsal and ventral channels.

Babylonia spirata (Linnaeus, 1758)
Figures 1C-F, 4D, 7D-F, 8A-B

Material examined: ZMMU Lc-24965, Tamil Nadu, in vicinities of Rameswaram and Tuticorin, India. Obtained from fishermen. October 2000, coll. Yu. Kantor. 1 ♀ 2 ♂ specimens dissected.

Protoconch: The protoconch of *B. spirata* (Fig. 1 D,E) is smooth, paucispiral and conical, with an initial diameter of about 170 µm. The transition from protoconch to teleoconch could not be unambiguously distinguished in any of the ten specimens with an uneroded protoconch that we examined. The sutural canal first appears at about 1¼ whorls (diameter of about 670 µm), suggesting that the larvae may hatch at this size. The color of the protoconch is dark reddish brown, gradually fading to ivory between the second and fourth whorl.

Shell ultrastructure: (Fig. 1 F). The shell of *B. spirata* is about 470 µm thick, and covered by a thick, brown, periostracum composed of closely spaced lamellae. The shell is composed almost entirely of comarginal crossed-lamellar crystals, with only a very thin (≈28 µm) outermost prismatic layer.

Anatomy: The anatomy of *B. spirata* is very similar to that of *B. areolata*, with the following minor differences. The glandular folds along the mid and posterior oesophagus of *B. spirata* were grayish rather than yellow, and the general arrangement of folds was more similar to *B. papillaris* than to *B. areolata*. The radular diverticulum is shorter than in *B. areolata* and occupies ~⅓ of retracted proboscis length. The accessory salivary gland is yellowish, larger, shorter, and dorso-ventrally flattened (Fig. 4D), spanning less then half of proboscis length. The longitudinal fold in the stomach is less pronounced than in *B. areolata,* but much more distinct than in *B. papillaris*. Ciliary currents in stomach were similar to those in *B. areolata.* The rectal gland is absent in *B. spirata.* Radula triserial, 5.5 mm long, 0.72 mm wide (0.014 SL), composed of 29 rows of teeth, of which 3 are nascent (Fig. 7D-F). Rachidian teeth with 3 long, closely spaced, cusps concentrated at middle of tooth, central cusp shortest, strongly buttressed along posterior margin of basal plate (Fig. 7F). Single, shorter, narrower cusps along outer edges of rachidian. Median cusp with sharp indentation to accommodate central cusp of anteriorly adjacent tooth (Fig. 7E-F). Lateral teeth bicuspid, with outer cusp > 2 times longer than inner cusp.

One of the radulae examined was unusually short, minute, with anteriormost teeth smaller than teeth on central and posterior portions of radula, suggesting possibility of amputation and subsequent regeneration of portion of buccal mass and radula (Carriker et al. 1972).

Of the two female specimens dissected, one had a fecund ovary above the digestive gland, the other possessed imposex features, including a short penis together with an incompletely formed *vas deferens*. A mature male specimen had a large yellow-orange testis that spanned the posterior surfaces of the uppermost 1.5 whorls of the visceral mass. A seminal duct (Fig. 8A, sem.d) runs anteriorly from the upper part of the testis along the inner wall of the visceral mass, receiving 7-8 coiled ducts from the testis (Fig. 8A, td). This duct enters the mantle cavity without forming a discernible seminal vesicle. After descending to the floor of the mantle cavity, it expands to form a prostate gland prior to reaching the base of the penis. The duct remains closed along its entire length. In a living animal, the penis is long, thin, tapering, flagellum-like, lacking a papilla. When this animal was preserved, the penis (Fig. 8B, p) contracted to become short, stout, and conical.

Babylonia lutosa (Lamarck, 1822)
Figure 1G, 9A-B

Material examined: ZMMU Lc-25237, Mirs Bay, Hong Kong, coll. J.D. Taylor.
1 ♂ +1 ♀ specimen dissected.

Anatomy of *B. lutosa* is, in all respects, very similar to that of *B. areolata*. Alimentary system differed from that of *B. areolata* only in that oesophagus broadened not immediately posterior to nerve ring, but at some distance from it. The dorsal glandular folds in this broadened region of the oesophagus are fused together, forming thick glandular lining. A rectal gland is absent. Gross morphology of the reproductive system as of a fecund female was the same as those of *B. areolata* and *B. papillaris*. Male reproductive system was the same as that of *B. spiralis*, except for the absence of the multiple coiled ducts entering the seminal duct.

Radula triserial, 12 mm long, 1.33 mm wide (0.022 SL) (female with SL ~ 60 mm), composed of 45 rows of teeth, of which 3 are nascent (Fig. 9A-B). Rachidian teeth with 3 long, closely spaced, cusps concentrated at middle of tooth, central cusp slightly shorter than flanking cusps, strongly buttressed along posterior margin of basal plate, indented to accommodate central cusp of adjacent tooth. Additional shorter, narrower, single cusps at outer margins of basal plate. Lateral teeth bicuspid, with outer cusp > 2 times longer than inner cusp. Central cusp broken on 7 consequent rachidian teeth at bending plane of radula.

Babylonia japonica **(Reeve, 1842)**
Figure 1D, 7A-C, 8C-D

Material examined: USNM 905325, Seafood market, Osaka, Japan. 1 ♂ +1 ♀ specimen dissected.

Anatomy of *B. japonica* is very similar to that of *B. areolata*. Rectal gland present, small, grayish, opens directly in mantle cavity outside rectum. Male reproductive system characterized by presence of normal long *vesicula seminalis*, situated on the border between testis and digestive gland (Fig. 8C). Radula triserial, 11.7 mm long, 1.84 mm wide (0.027 SL) (female with SL 68.0 mm), composed of 42 rows of teeth (Fig. 7A-C).
Rachidian and lateral teeth similar to those of *B. lutosa*.

Babylonia zeylanica **(Bruguière, 1789)**
Figure 1I, 9C-D

Material examined: ZMMU Lc-25173, South India, off Tuticorin, from fishermen, 1 ♀ specimen (SL 60.7 mm), radula examined.

The preservation of the single specimen available was poor. Gross external anatomy was identical to that of other *Babylonia*, and is figured by Riedel (2000). We were not able to determine if this species has a rectal gland or accessory salivary gland based on the specimen available to us.
Radula triserial, 10.9 mm long, 1.49 mm wide (0.025 SL), composed of 37 transverse rows of teeth, of which 3 nascent (Fig. 9C-D). Rachidian teeth similar to those of *B. lutosa*, but basal plate slightly narrower, with outermost cusps closer to central group of 3 cusps. Lateral teeth also similar to those of *B. lutosa*, but outer cusps slightly shorter and broader.

Phylogenetic Analyses Of Morphological Data

A maximum parsimony analysis of the morphological data using the Exhaustive Search Option of PAUP 4.02b (Acctran character optimization) yielded a single most parsimonious tree [L = 62, Consistency index (CI) = 0.613, Retention Index (RI) = 0.671] shown if Figure 10A. When the analysis was repeated using Deltran character optimization, a single tree was produced with identical topology, length and indices, but differing slightly in the character optimization and branch lengths within the non-buccinoidean clade. Only the node uniting the two representative species of *Babylonia* enjoyed significant bootstrap or jackknife support. The remaining nodes uniting rachiglossan neogastropods had Bremer support values of 1 and lacked bootstrap or jackknife support.

Of the 24 anatomical characters (comprising 62 states) used in this study, 15 exhibited some degree of homoplasy, and 2 of the remaining 9 were autapomorphic and therefore parsimony uninformative.

Phylogenetic Analyses Of Molecular Data

Maximum parsimony analysis [Branch and Bound Search] of the aligned 591 base pair sequences resulted in a single most parsimonious tree [L = 1065, CI = 0.409, RI = 0.383] shown in figure 10B. There was significant bootstrap and jackknife support only for the monophyly of *Busycon*, Busyconinae, *Neptunea*, Fasciolariidae and Muricidae. Of the 591 characters, 224 were parsimony-informative.

DISCUSSION

The genus *Babylonia* has traditionally been attributed to the family Buccinidae, primarily on the basis of its "bucciniform" shell shape with very short anterior canal, supplemented by the inferences of similarity in external anatomy (see Reeve, 1849) and radular morphology (based on line drawings of radulae produced using light microscopy).

The Buccinoidea, comprising the families Buccinidae, Fasciolariidae, Melongenidae, Nassariidae, Columbellidae and Colubrariidae, is generally regarded as a morphologically cohesive, monophyletic group within Neogastropoda (e.g., Ponder, 1974; Ponder & Warén, 1988; Kantor, 1996; Harasewych et al., 1997b). Although there is little agreement as to the rank or inter-relationships of its constituent higher taxa (e.g. Thiele, 1929; Wenz, 1938; Ponder & Warén, 1988; Kantor, 1996), buccinoideans share a number of anatomical features, among them: a long proboscis with a terminal buccal mass; a short radular diverticulum with the odontophore protruding into the buccal cavity; a radula with bicuspid to multicuspid lateral teeth and with rachidian teeth composed of a flattened basal plate without cusps or with cusps emanating at an acute angle from its mid- to posterior region; the absence of accessory salivary glands; and the absence of a rectal gland. Most Buccinoidea have a well-developed valve of Leiblein and large gland of Leiblein, although one or both of these structures have been lost

in some taxa [e.g. in the subfamily Volutopsiinae (KANTOR, 1990) and the genus *Melongena* (PONDER, 1974)]. The buccinoidean mid-esophagus lacks glandular dorsal folds, although it may become secondarily glandular in Colubrariidae (PONDER, 1968). The buccinoidean stomach is extremely variable. A posterior mixing area can be present (sometimes very large and long, as in Nassariidae), or absent. A gastric shield is developed in some Nassariidae, Columbellidae and Buccinidae, but is absent in most genera. Ducts of digestive gland can be paired and broadly separated, closely spaced, or fused into a single duct prior to entering the stomach.

The gross morphology of the animal, mantle cavity, and radula of species of *Babylonia* have led several authors to infer or accept the affinities of this genus to Buccinidae (KIENER, 1835; ADAMS & REEVE, 1848; EYDOUX & SOULEYET, 1852; SOWERBY, 1902; ALTENA & GITTENBURGER, 1983; RIEDEL, 2000). However, our more detailed examination of the anatomy of the digestive system of *Babylonia* revealed it to differ substantially from that of all buccinoideans studied to date. All species of *Babylonia* have a single, unpaired accessory salivary gland with typical neogastropod histology (Fig. 4D), consisting of two layers of epithelium (Fig. 4D, oel, iel) separated by a layer of circular muscle fibers (Fig. 4D, cml). Both the valve of Leiblein and the gland of Leiblein are absent in all species of *Babylonia* studied. Posterior to the nerve ring, the oesophagus abruptly expands, its dorsal wall occupied by enlarged, conspicuously glandular folds (Fig. 6H). The salivary glands, nerve ring, and posterior esophagus are enveloped in dense connective tissue, a feature not known in any buccinoidean.

Scanning electron micrographs reveal the radula of *Babylonia* to differ consistently from the radulae of Buccinoidea. While the lateral teeth of *Babylonia* are very similar in shape to those of some Buccinidae and Melongenidae, the rachidian teeth are of fundamentally different design. In *Babylonia*, the bases of the three central cusps emanate from the anterior edge of the basal plate and are strongly buttressed (see Fig. 7F), being similar in this respect to the rachidian teeth of *Oliva*, Muricidae, and some
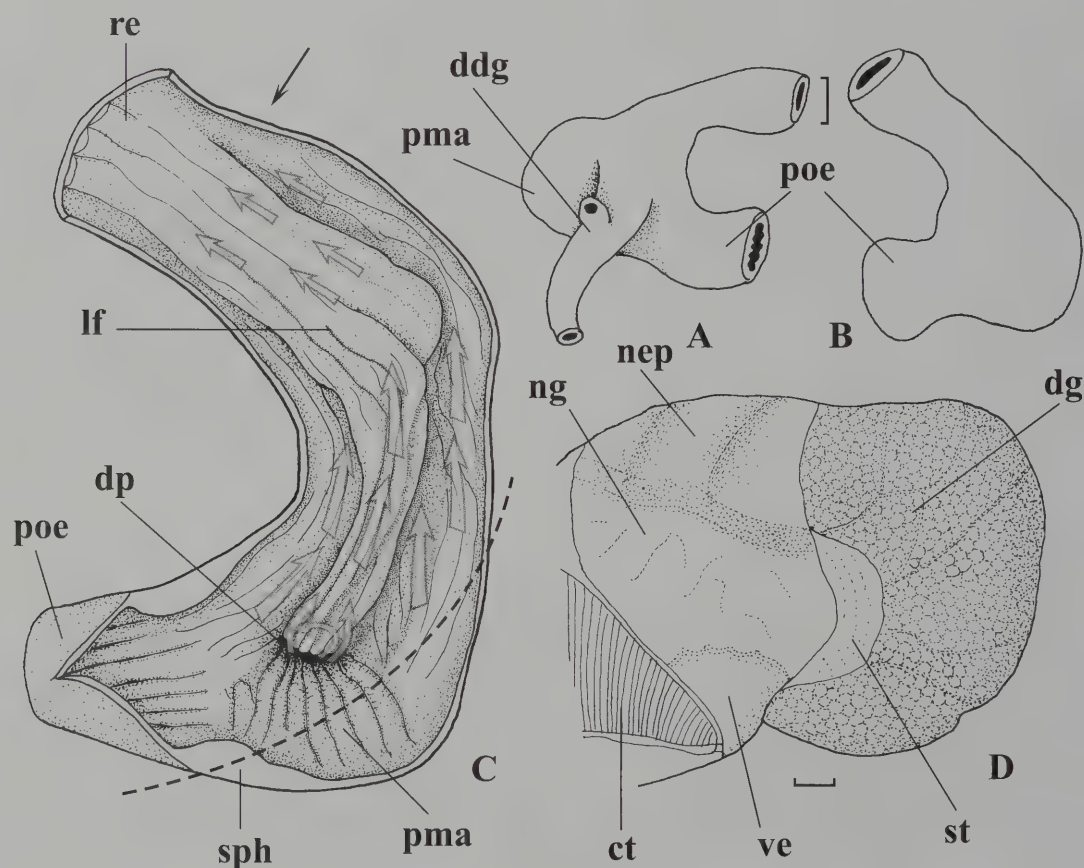
Figure 3. Anatomy of the stomach of *Babylonia areolata* (Link, 1807). **A**— Dorsal and **B**— ventral views of the stomach. **C** — Stomach opened along dorsal midline. Open arrows indicate ciliary currents. The dashed line represents the edge of the nephridium and nephridial gland, while the black arrow demarcates the posterior limit of the mantle cavity. **D** — View of the visceral mass showing the stomach nearly completely covered by the nephridium. Scale bars = 1 mm.

Abbreviations: ct, ctenidium; ddg, duct of the digestive gland; dg, digestive gland; dp, pouch of the duct to the digestive gland; lf, longitudinal fold of the stomach; nep, nephridium; ng, nephridial gland; pma, posterior mixing area; poe, posterior oesophagus; re, rectum; sph, sphincter between the posterior oesophagus and stomach; st, stomach; ve, ventricle.
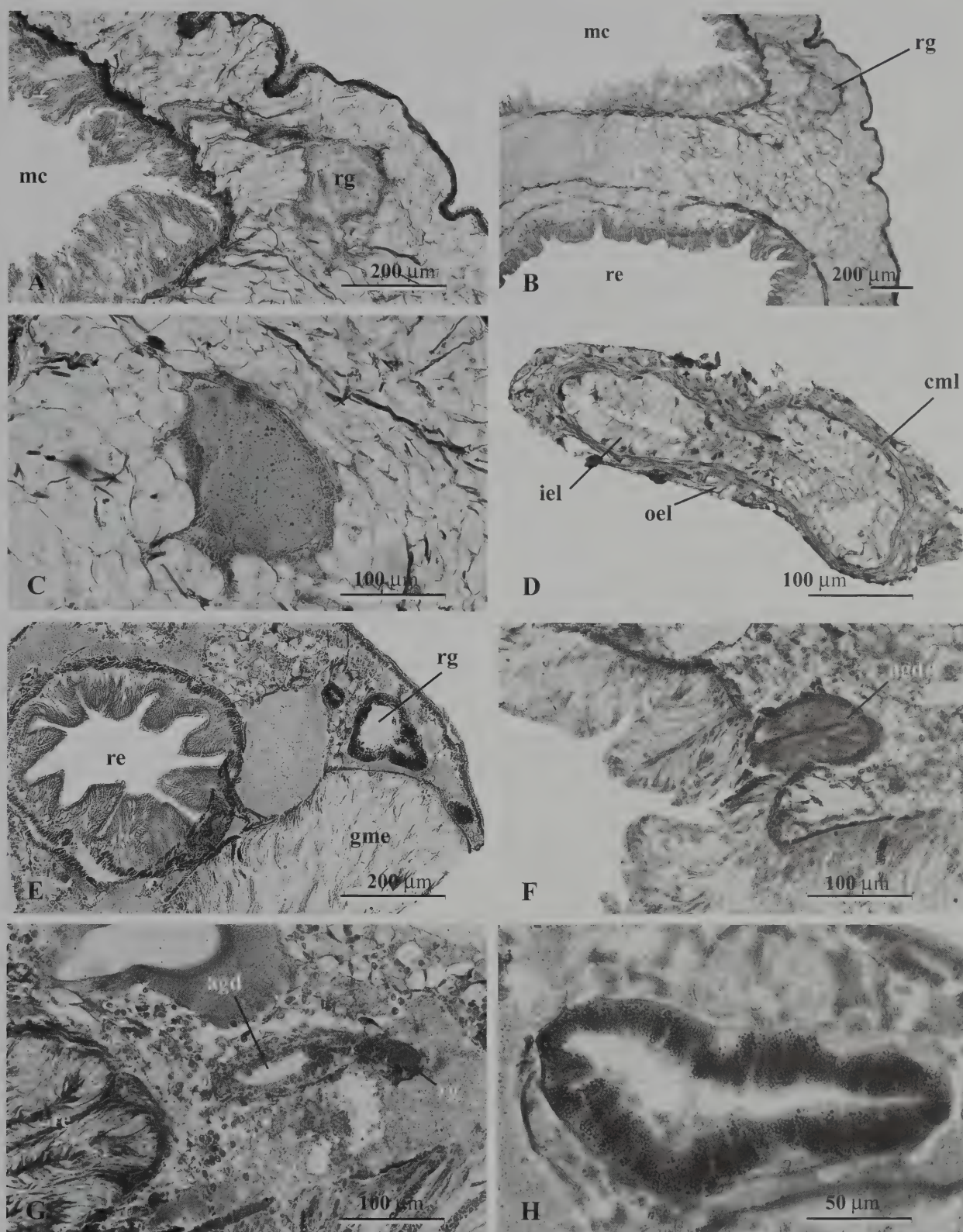
Figure 4. Histology of rectal gland and accessory salivary gland. **A** — Transverse section through posterior part of mantle of *B. areolata*, showing rectal gland opening into the mantle cavity. **B** — Same, at lower magnification, showing position of rectum. **C** — Transverse section through rectal gland of *B. areolata* at midlength. **D** — Transverse section through accessory salivary gland of *B. spirata*. **E-H** — Rectal gland of the conoidean *Horpospira maculosa*. **E** — transverse section through posterior part of mantle to show the rectal gland and rectum. **F** — Opening of the rectal gland duct into the mantle cavity. **G** — Transverse section through the rectal gland at the transition of the gland into the duct (explanation in the text). **H** — transverse section of the rectal gland.

Abbreviations: cml, circular muscle layer; gme, glandular mantle epithelium; iel, inner epithelial layer; mc, mantle cavity; oel, outer epithelial layer; re, rectum; rg, rectal gland; rgd, duct of the rectal gland.

Volutidae. The smaller, flatter cusps along the outer edges of the rachidian teeth of *Babylonia* somewhat resemble the "marginal cusps" found is some muricids (see Kool, 1987).

One of the most prominent characters of *Babylonia* is an extremely shortened digestive system. The stomach is nearly completely covered by the nephridium and only part of the posterior mixing area is visible beyond the posterior margin of the nephridium. The stomach is greatly simplified and small,
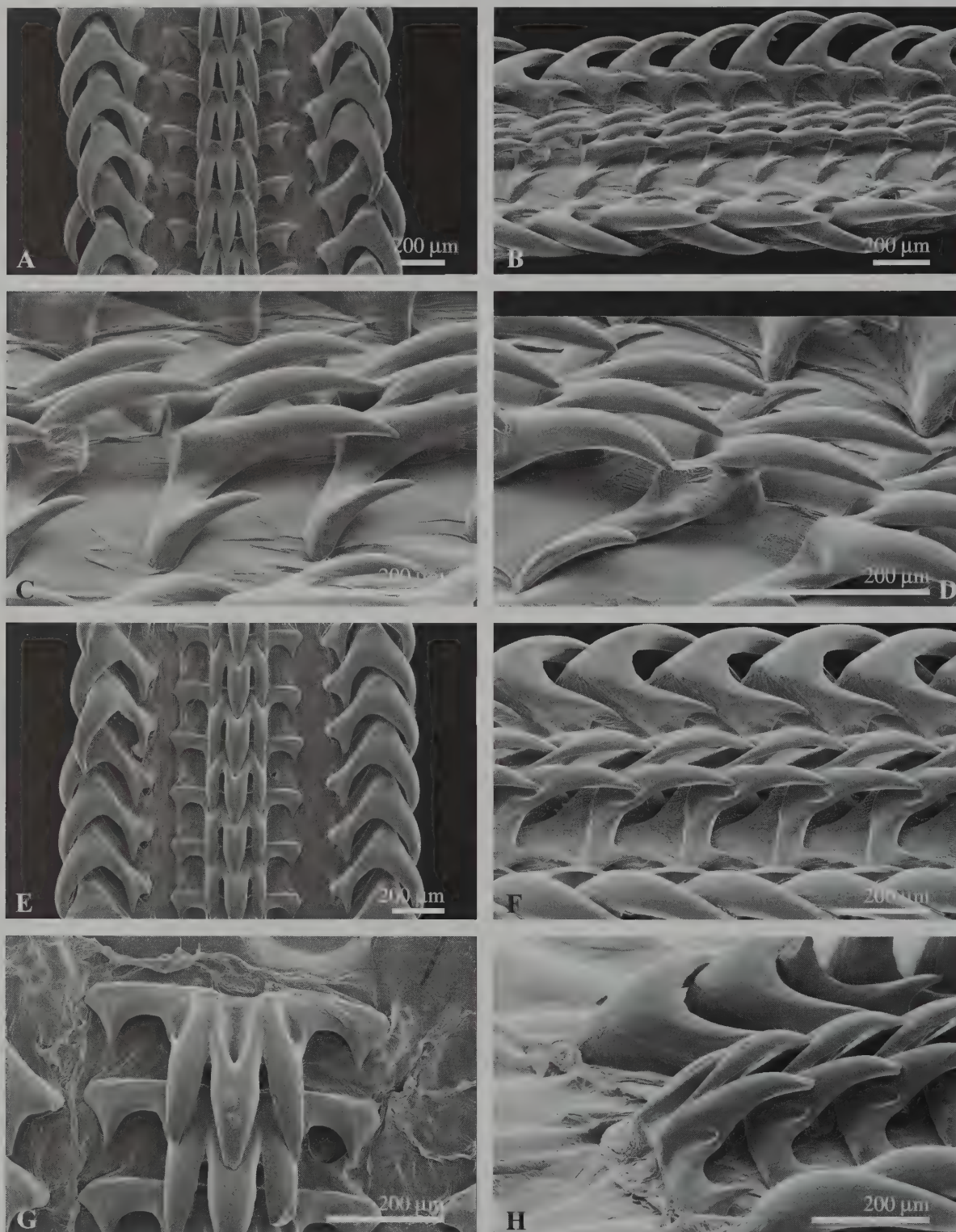
Figure 5. Radulae of *Babylonia areolata* (**A-D**) (SL 38.0 mm) and *Babylonia papillaris* (**E-H**) (SL unknown). **A, E** — Dorsal and **B-E** — left lateral (45°) views of central portion of radular ribbon. **C,D** — left lateral (45°) views of rachidian to show indentation (**C**) and buttress of central cusp (**D**). **G,H** — dorsal and left lateral views of posteriormost rachidian teeth to show 3 indentations.

with a very short, muscular caecum. The juncture of the posterior oesophagus and stomach is marked by large and powerful sphincter, which is bright red in living specimens. The stomach is not divided into dorsal and ventral channels, and may have either a strong longitudinal fold (*B. areolata, B. spirata*) or smaller longitudinal folds (*B. papillaris*). The intestine is indistinguishable anatomically, and the stomach appears to open directly into the rectum. The ducts of the digestive
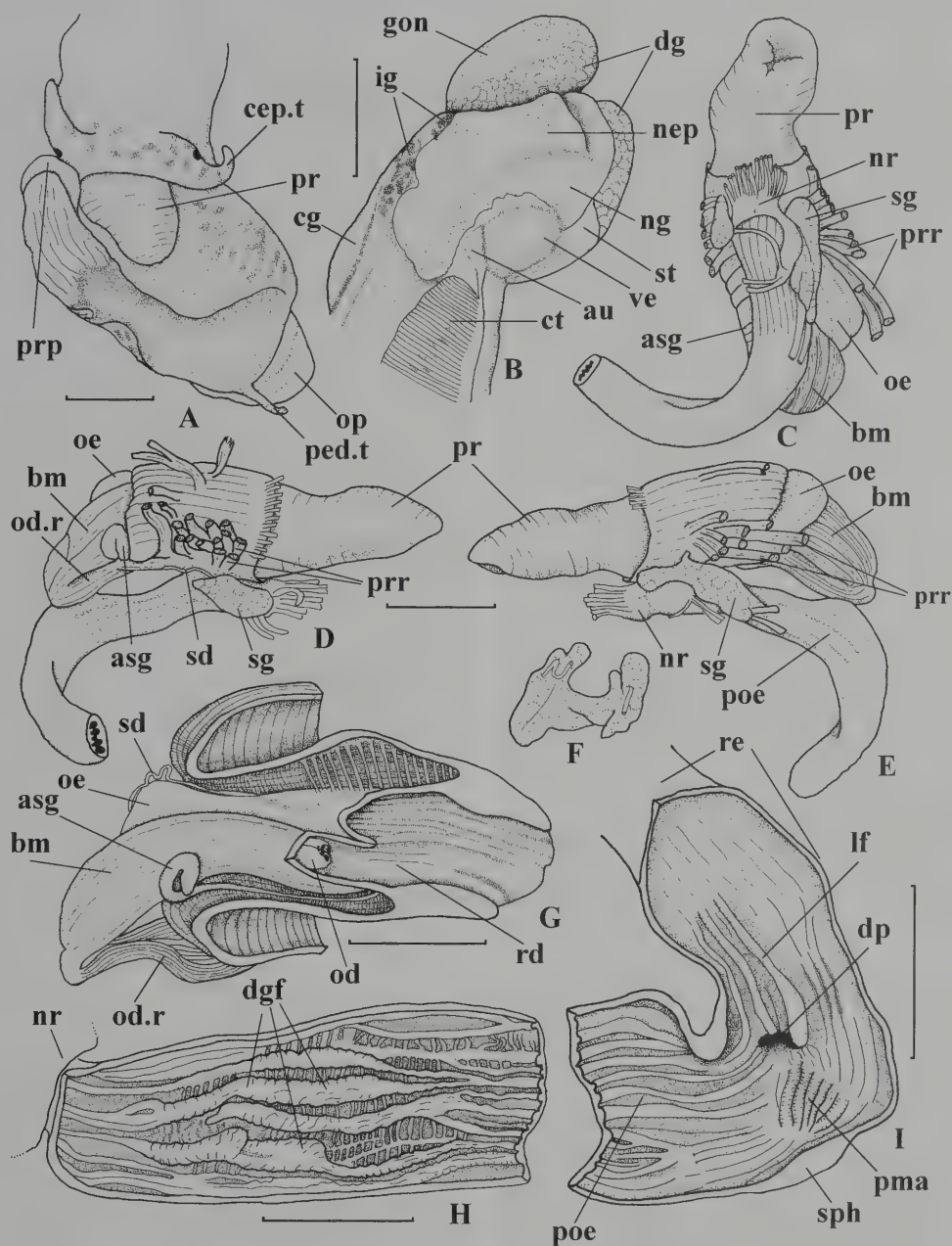
Figure 6. Anatomy of *Babylonia papillaris* (Sowerby, 1825). **A** — Head-foot of animal removed from the shell. **B** — View of the visceral mass to show the stomach, nearly completely covered by nephridium. **C** — Ventral, **D** — right lateral, and **E** — left lateral views of anterior alimentary system. **F** — Dorsal view of the salivary gland. **G** — Proboscis opened from the right side. **H** — Mid- posterior oesophagus, opened ventrally to show the dorsal glandular folds. **I** — Stomach opened along midline of the dorsal wall. Scale bars = 5 mm.

Abbreviations: asg, accessory salivary gland; au, auricle; bm, buccal mass; cep.t, cephalic tentacle; cg, capsule gland; ct, ctenidium; dg, digestive gland; dgf, dorsal glandular folds of the oesophagus; dp, pouch of the duct of digestive gland; ig, ingestive gland; gon, donade; lf, longitudinal fold of the stomach; nep, nephridium; ng, nephridial gland; nr, circumoesophageal nerve ring; od, odontophore; od.r, odontophore retractor; oe, oesophagus; op, operculum; ped.t, pedal tentacle; pma, posterior mixing area; poe, posterior oesophagus; pr, proboscis; prp, propodium; prr, proboscis retractor muscles; rd, radular diverticulum; re, rectum; sd, salivary duct; sg, salivary gland; sph, sphincter between the posterior oesophagus and stomach; st, stomach; ve, ventricle.

gland join prior to entering the stomach via a single opening.

The tubular rectal gland, found in *Babylonia areolata* and *B. japonica*, runs along the rectum but opens into the mantle cavity (Fig. 4B) rather than into rectum, as in other neogastropods. This gland can be distinguished from the surrounding tissues by its darker coloration, but its epithelium is low and poor in melanin granules, unlike that of *Nucella lapillus*, the only species for which the rectal gland has been examined histologically (FRETTER & GRAHAM, 1962; ANDREWS, 1992). The differences between the rectal gland of *Babylonia* and those of most other neogastropods raise questions as to the homology of these structures. The rectal gland of *Hormospira maculosa* (Pseudomelatomidae, Conoidea) is intermediate in morphology between *Babylonia* and *Nucella* in that it opens into the mantle cavity very near the anus (KANTOR, 1988) (Fig. 4F, agd). In this species, the posterior portion of the gland is lined with tall
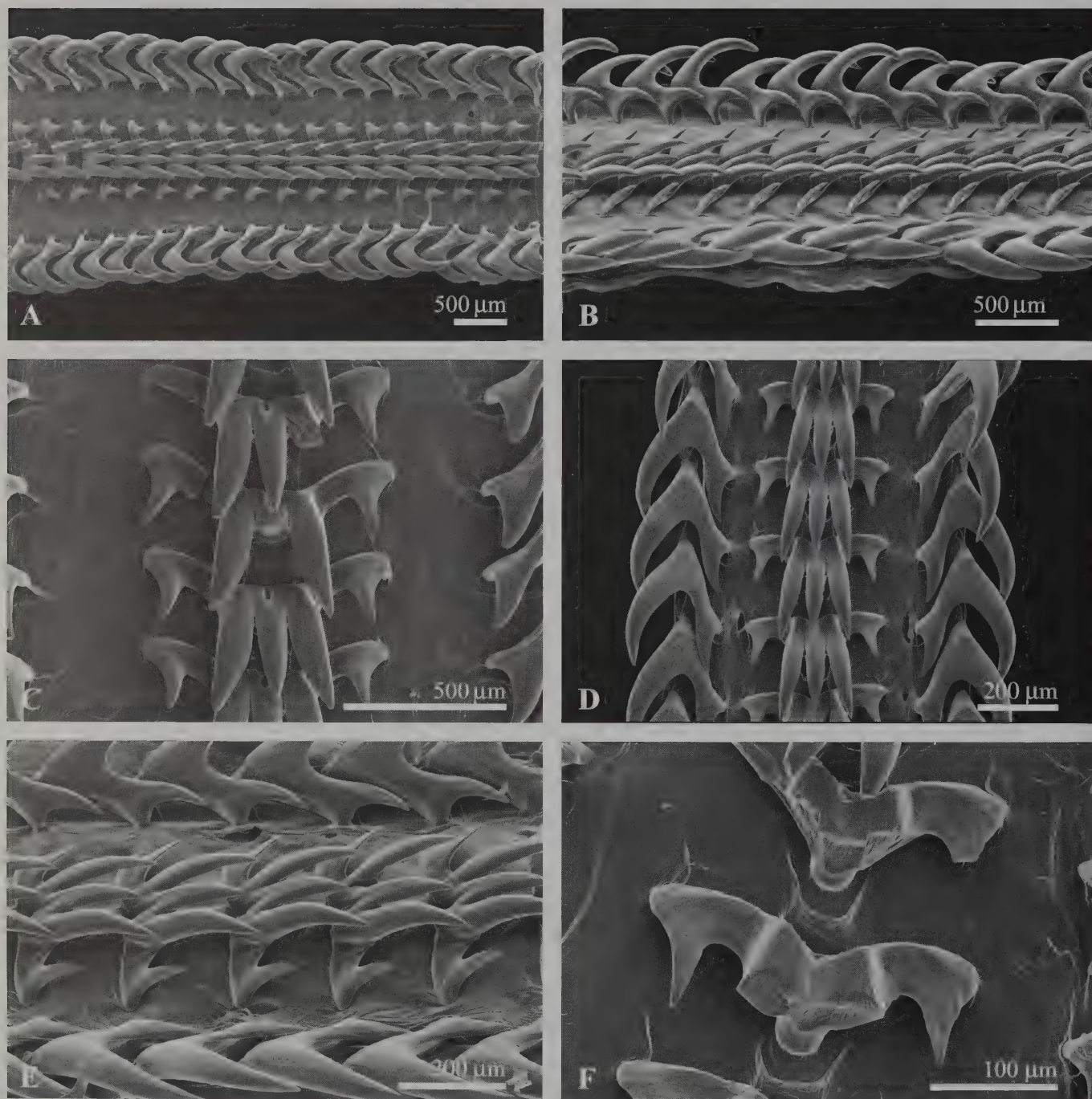
Figure 7. Radulae of *Babylonia japonica* (**A-C**) (SL 68.0 mm) and *B. spirata* (**D-F**) (SL 53.0 mm). **A, D** — Dorsal and **B-E** — left lateral (45°) views of central portion of radular ribbon. **C** — dorsal enlarged view of rachidian teeth to show the indentation at base of median cusp. **F** — dorsal enlarged view of damaged rachidian teeth from bending plane to show indentation and buttress of central cusp.

epithelial cells containing melanin granules (Fig. 4H, similar to *Nucella*), while the anterior region forms a duct (Fig. 4G, agd) lined with lower epithelial cells containing reduced quantities of melanin granules. The epithelium in the anterior region of the rectal gland of *Hormospira* is similar to, and likely homologous with, the rectal gland of *Babylonia* (Fig. 4C).

Dissection of a single male specimen of *Babylonia spirata* revealed that the seminal duct does not form a seminal vesicle, but rather is joined by numerous, coiled ducts originating in the testis (Fig. 8A, td). The single, poorly preserved male specimen of *B. lutosa* available for study lacked these ducts as well as a seminal vesicle. In male specimens of *B. japonica*, the seminal duct does form a well-defined seminal vesicle (Fig. 8C, vs).

Phylogenetic analyses of morphological (Fig. 10A) as well as molecular (Fig. 10B) data sets reveal *Babylonia* to be more closely related to volutoidean families such as Volutidae, Turbinellidae and Olividae than to any member of the Buccinoidea. The high incidence of homoplasy in morphological characters that has long confounded attempts to resolve phylogenetic relationships within Neogastropoda (e.g., Ponder, 1974; Kantor, 1996) is also apparent in this study. While the morphological tree is fully resolved, only the node uniting species of *Babylonia* has significant bootstrap, jackknife or Bremer support. Moving the two species of *Babylonia* to a basal position within Buccinoidea increased the tree length by 4 steps (6%), while including *Babylonia* at various nodes within Buccinoidea increased tree length by as little as 2 steps (3%) (sister taxon to *Melongena*) to as many as 5 steps (8%) (sister taxon to either *Buccinum*, *Neptunea* or *Chlanidota*).

Portions of the phylogeny based on partial sequences of the cytochrome c oxidase subunit I mitochondrial gene are slightly
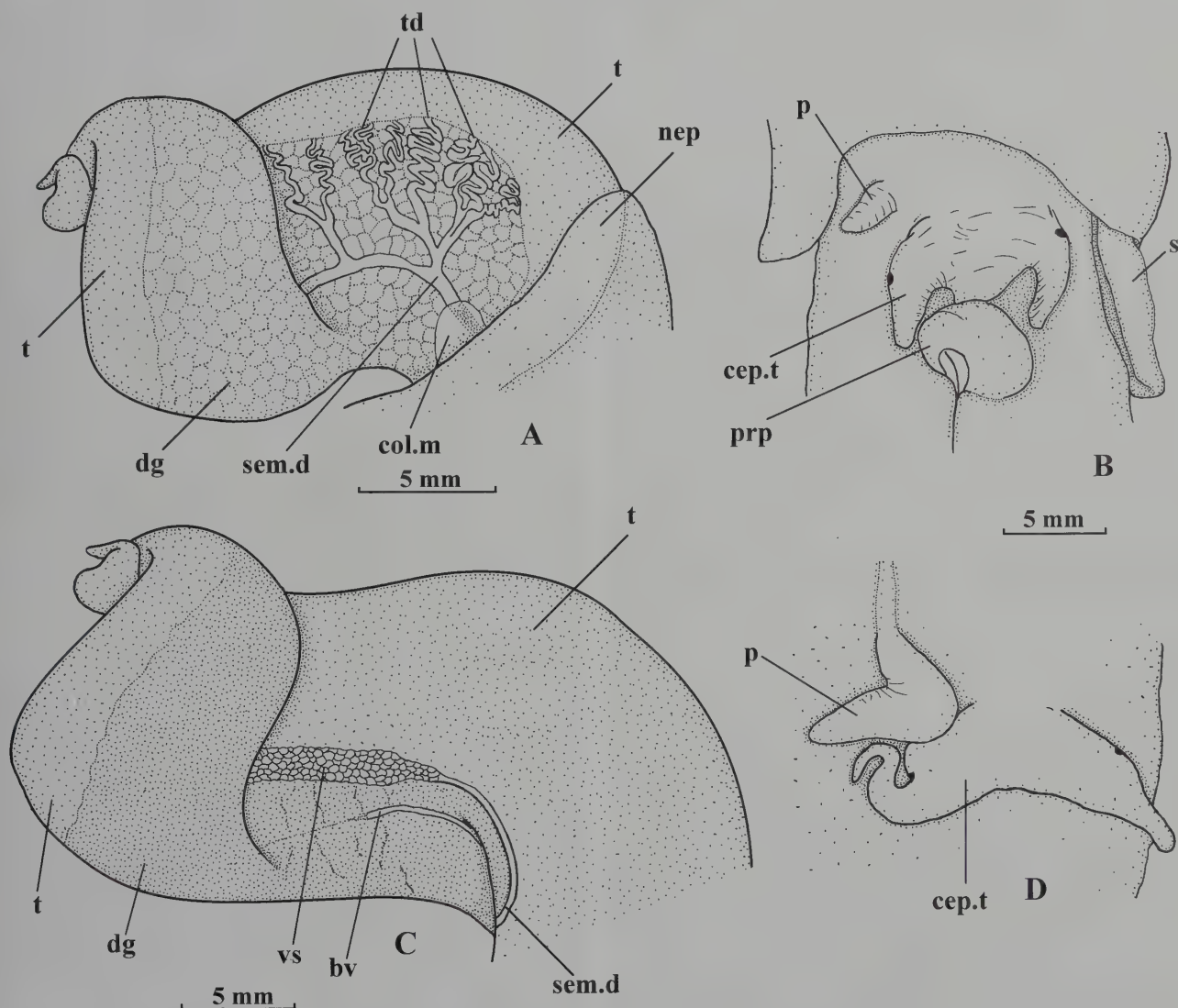
Figure 8. Male reproductive system of *Babylonia*. **A-B**. *Babylonia spirata*. **C-D**. *Babylonia japonica* (Reeve, 1842). **A,C** – view of internal part of the whorls of visceral mass to show position of seminal duct and *vesicula seminalis* (**C**). **B, D** — anterior views of foot-head to show penis.
Abbreviations: bv, blood vessel; cep.t, cephalic tentacles; col.m, columellar muscle; dg, digestive gland; nep, nephridium; p, penis; prp, propodium; s, siphon; sem.d, seminal duct; t, testis; td, ducts from testis to seminal duct; vs, *vesicula seminalis*.

more robust, in that there is significant support for the monophyly of the families Fasciolariidae, Muricidae and the subfamily Busyconinae. Thirteen additional steps (6% of phylogenetically informative characters) are required to shift *Babylonia* to the base of Buccinoidea, while placing the genus within Buccinoidea requires 19-31 additional steps (9-14% of phylogenetically informative characters). Although neither morphological nor molecular data provide robust support for the monophyly of Buccinoidea, both data sets exclude *Babylonia* from the Buccinoidea, indicating instead affinities with the "volutoidean" families Volutidae, Turbinellidae and Olividae. As our analyses contain a small proportion of the families historically attributed to "Volutoidea" [show to be grade rather than a clade by HARASEWYCH ET AL., 1997b], identification of the sister group of *Babylonia* must await further study. While we are not suggesting that species of *Babylonia* are congeneric with the olivid genus *Eburna*, it appears that LAMARCK (1822) was perhaps more insightful than many of his successors in recognizing the relationship between these taxa.

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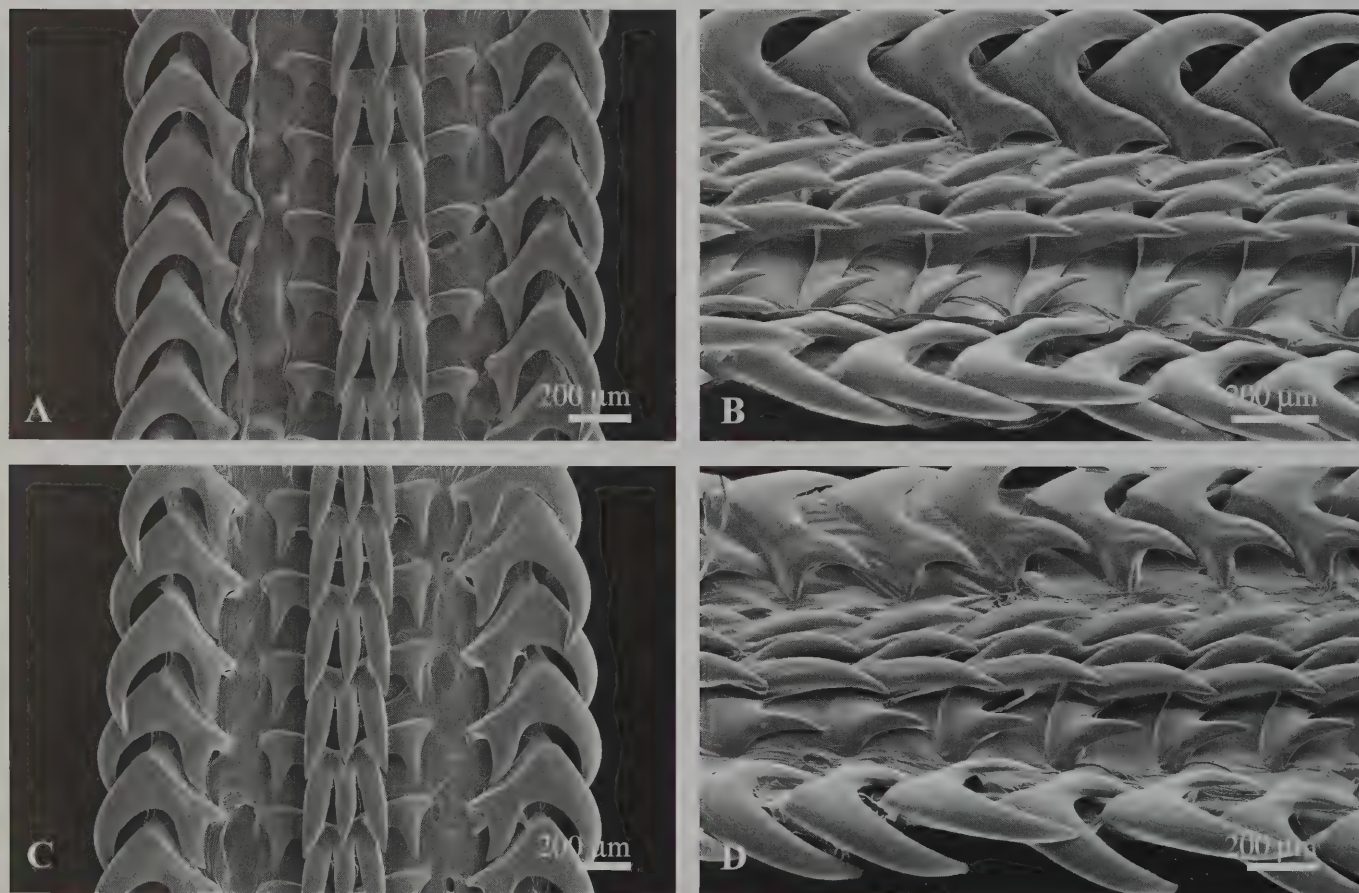
Figure 9. Radulae of *Babylonia lutosa* (A-B) (SL ~60 mm) and *B. zeylanica* (C-D) (SL 60.7 mm). **A, C** — Dorsal and **B-D** — left lateral (45°) views of central portion of radular ribbon.

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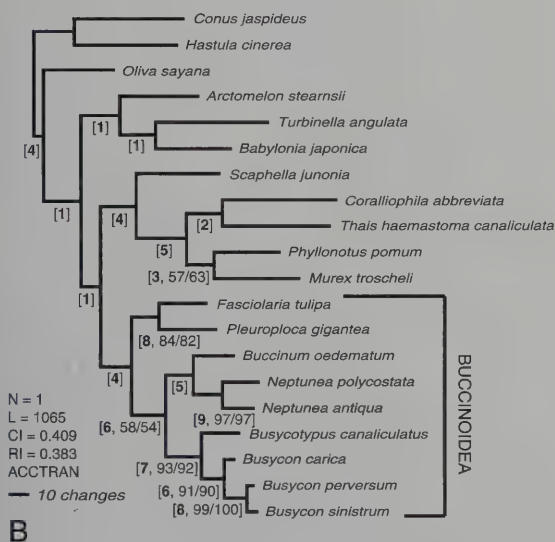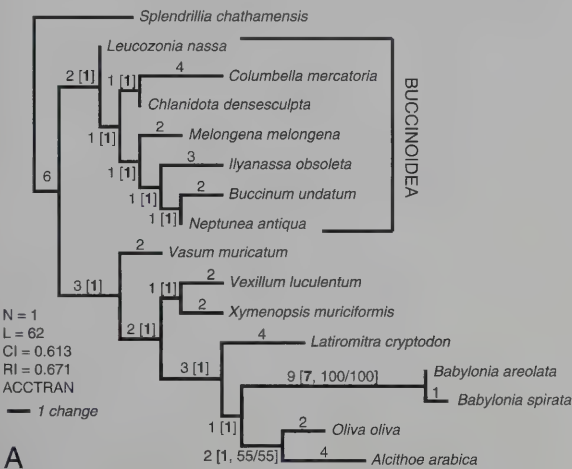
Figure 10. Phylogenetic relationships of *Babylonia* to rachiglossan Neogastropoda. **A** — The single most parsimonious tree resulting from a maximum parsimony analysis (branch and bound search, ACCTRAN character transformation) of the anatomical data matrix (Table 1). Branch lengths are shown on all nodes. Square brackets [ ] enclose Bremmer support indices (**bold**) followed by bootstrap / jackknife proportions in percent, for the nodes for which support exceeded 50%. **B** — The single most parsimonious tree derived from a maximum parsimony analysis (branch and bound search) of partial cytochrome c oxidase I sequences (591 bp) of taxa in Table 2. Square brackets [ ] enclose Bremmer support indices (**bold**) followed by bootstrap / jackknife proportions in percent, for the nodes for which support exceeded 50%.

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Comparative biology of *Conus* in the light of phylogeny: a preliminary report

Alan J. Kohn

KEY WORDS: *Conus*, comparative ecology, comparative development, phylogeny.

ABSTRACT In this paper I summarize past interspecific comparative studies of *Conus*, and indicate how interpretation of the results differs in the absence of phylogenetic information from that now possible with a recently available phylogenetic hypothesis based on molecular genetic data. I first briefly review the origin of *Conus*, including when, where and from what ancestors the genus arose, and its subsequent evolutionary history, then summarize the results of comparative analyses of food and habitat resource use, and I examine whether these patterns, as well as those of larval development reflect a phylogenetic signal.

RIASSUNTO In questo articolo riporto una revisione dei passati studi di comparazione interspecifica su *Conus*, ed indico come l'interpretazione dei risultati differisce in assenza di informazioni filogenetiche. Tali informazioni sono ora disponibili anche grazie alle ipotesi filogenetiche recentemente proposte sulla base di dati molecolari.
Esamino inizialmente l'origine del genere *Conus*, includendo quando, dove e da quale antenato il genere è sorto, e la sua successive storia evolutiva. Quindi passo in rassegna i risultati delle analisi comparative su alimentazione e uso delle risorse di habitat, ed esamino se questi pattern, così come quelli sullo sviluppo larvale, riflettano un segnale filogenetico.

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INTRODUCTION

The Neogastropoda that this symposium celebrates contains the largest and most widely distributed genus of marine molluscs and probably of marine invertebrates, *Conus*, a taxon with several unusual attributes: 1) Its radular structure and function differ from those of other molluscs in that each radular tooth functions independently as a hollow hypodermic needle that is injected into prey organisms. 2) Conotoxins, small powerfully neurotoxic peptides, are injected through the radular tooth to overpower the prey. 3) The shape and structure of the shell differs markedly from those of its closest relatives; it is conical or biconic with a thick, strong outer whorl but with inner walls nearly completely dissolved.

Conus is a particularly important genus, especially in tropical regions, for several reasons: 1) It is the most diverse genus of marine molluscs, with more than 500 extant species, and very large numbers of species often co-occur in the same habitat, enhancing marine biodiversity in these communities. 2) The genus is very widely distributed, and some species occupy the entire Indo-West Pacific region, an area comprising one-fourth of the world ocean. 3) It can be very abundant, up to 40/m^2 but usually two orders of magnitude less dense. 4) Because of its carnivorous habits and abundance, *Conus* is ecologically important, especially in coral reef-associated communities. 5) The high specificity of conotoxins for particular receptor proteins and their small size and ease of characterization and synthesis makes them particularly useful for neurobiological and medical applications. 6) The shells of *Conus* are durable and well preserved in the fossil record, since its first appearance in the Lower Eocene, about 55 million years ago. Its large number of species combined with its geologic youth indicate a higher rate of evolutionary diversification than for any other marine gastropod group (STANLEY, 1979).

In this report I briefly review three topics, the origin and subsequent evolutionary history of *Conus* to set the historical context, and two aspects of its comparative biology, use of habitat and food resources. All previous of comparative studies of *Conus* (e.g. KOHN, 1959, 1968, 1981, 1997, 2001; KOHN and ALMASI, 1993; KOHN & NYBAKKEN, 1975; NYBAKKEN, 1979) were constrained by having to treat all species as independent entities, because their phylogenetic relationships were unknown. Now for the first time, an objective, species-level phylogenetic hypothesis is available, based on the pioneering work of Thomas Duda, who has elucidated the nucleotide sequences of two genes in about 70 species (DUDA & PALUMBI, 1999; DUDA, KOHN & PALUMBI, 2001). We can thus begin to investigate how the similarity of species along ecological gradients relates to their phylogenetic affinities. In this preliminary account, I evaluate whether species that are more closely related according to the molecular phylogenetic hypothesis are more similar ecologically, that is with respect to their use of microhabitat types and prey species.

ORIGIN AND SUBSEQUENT EVOLUTIONARY HISTORY OF CONUS

The oldest known species is *Conus concinnus*, described by James de Carle Sowerby in 1821 from the Lower Eocene of England. *C. roualti* D'Archiac, 1850, described from France, is very similar and probably contemporaneous. An uncritical compilation of the paleontological literature of *Conus* (KOHN, 1990; from which most of the following account is summarized) indicated that the first real radiation of the genus occurred in the Middle Eocene and that by Late Eocene it had spread widely around the world. More than 100 *Conus* species are known from Eocene strata. Some persisted from early in the epoch, but 75% of Late Eocene species originated then. The prevailing warm ocean

temperatures and open Tethys Sea probably played roles in this diversification in species richness and enhanced geographic range. During the cooler Oligocene epoch, both the numbers of species and the geographic range of the genus contracted. Only about 70 species are known, and the northernmost representatives of the genus, in present Denmark and Washington State, became extinct. In the Miocene, *Conus* blossomed with a wealth of new forms. More than 300 Miocene species are known, and there were one or more major radiations. According to the fossil record, Europe continued to support the highest species richness of *Conus* in the Miocene. Then the seas cooled again, species richness dropped again, and the Indo-Pacific region became the center of again burgeoning diversity in the Pleistocene and Recent (KOHN, 1985). Overall, the temporal pattern of *Conus* diversity in the Cenozoic is a general increase, with times of rapid radiation punctuated by periods of reduced diversity. Extrinsic physical factors are the most likely causes of these patterns, because they closely parallel those shown by other gastropods and other invertebrate groups during Cenozoic time (e.g. RAUP, 1976).

Fig. 1. Number of species of *Conus* on subtidal complex reef platforms (solid diamonds) and intertidal smooth limestone benches (open circles) in the Indo-West Pacific region, plotted against longitude. For reef platforms (N=26), each point represents a census of 22-650 (mean=197) individuals. For benches (N=13), each point represents a census of 38-414 (mean=133) individuals. Data from KOHN (1967, 1971, 2001).

COMPARATIVE HABITAT RESOURCE USE

Conus species occupy many different marine environments from the intertidal zone to depths of nearly 600m, but most comparative data derive from studies of coral reef-associated habitats, different types of which support different numbers of co-occurring species. The extensive sandy bottoms of atoll and barrier reef lagoons typically harbor the fewest species (1-8; mean=3). Topographically smooth, environmentally harsh intertidal benches support 6-9 (mean 8), typically support a set of small (maximum shell length 20-30mm) species that is quite constant in species composition throughout the Indo-Pacific region. As has long been known (KOHN, 1967) topographically complex, subtidal coral reef platforms, with many different microhabitats, have the most diverse assemblages (9-36 species;

Fig. 2. Total *Conus* population density (A) and species richness (B) plotted against per cent cover of living coral. Both are negatively and highly significantly ($P<0.001$) correlated with living coral cover. A: r_s = -0.70; B: r_s = -0.59; N=54. Solid circles indicate data from Micronesia; open circles indicate data from Australian Great Barrier Reefs. Modified from KOHN (1983).
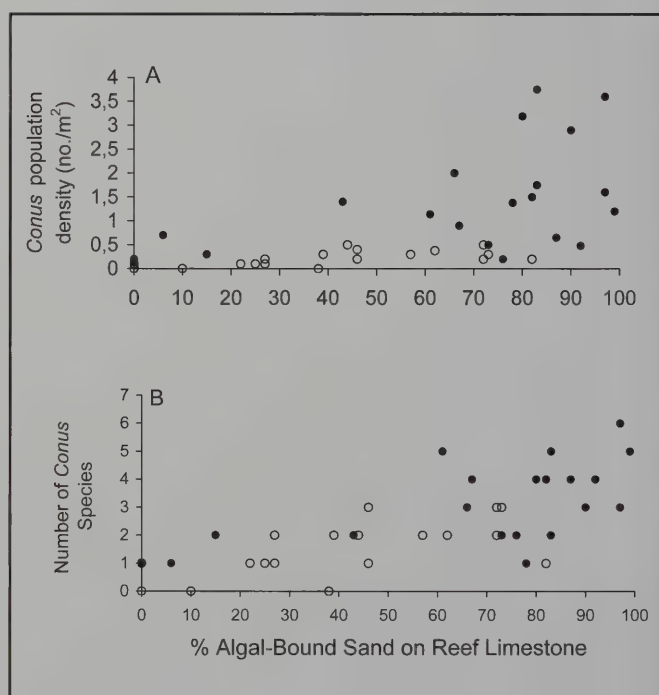
Fig. 3. Total *Conus* population density (A) and species richness (B) plotted against per cent cover of algal-bound sand on reef limestone. Both are positively and highly significantly ($P<0.001$) correlated with cover of algal-bound sand on reef limestone. A: r_s = 0.83; B: r_s = 0.84; N=54. Solid circles indicate data from Micronesia; open circles indicate data from Australian Great Barrier Reefs. Modified from KOHN (1983).

mean=16). Species richness is thus much more variable on reef platforms, and while species composition differs increasingly with distance in both, mean species turnover on reefs is 55% but only 39% on benches throughout the Indo-Pacific region (KOHN, 1997). There is also a strong longitudinal pattern of species richness on reefs, with maximum species richness in Papua New Guinea, declining to both the east and west, but no such pattern on benches (Fig. 1).

These striking between-habitat differences in *Conus* species richness also extend to within-habitat use of different microhabitats on coral reef platforms. These are complex, biogenic mosaics of different microhabitats, where both species richness and abundance reflect the availability of specific substrate types. Based on a series of transect studies that measured abundance and diversity of all *Conus* species collectively relative to available microhabitats (KOHN, 1983), the substrate on the reef favored least by *Conus* is living coral (Fig. 2). Its nematocysts sting the bare feet of the gastropods and thus deter treading on it, and it is an unfavorable microhabitat for prey organisms of *Conus* as well. The most favored substrate is a layer of sand bound by filamentous algae on reef limestone, favorable both for shelter and food (Fig. 3).

Also within habitats, different *Conus* species vary both with respect to specific microhabitat types occupied and along the gradient between specialized and generalized use of these types. Fig. 4 illustrates how frequently different *Conus* species are found on different substrates, using the 12 species with the largest samples as examples. The most commonly used substrate types are listed at the right, arranged from the softest, in the foreground, to the hardest. Species are arranged from specialists on soft substrates at the left, through generalists toward the center, to specialists on hard substrates at the right. The histograms clearly show gradients of substrate type use: for example, *C. litteratus* and *C. leopardus* are specialists found almost exclusively on large patches of sand on reef platforms, *C. coronatus*, also on sand but typically in small pockets in reef limestone, *C. pennaceus*, on sand under coral rocks, and *C. rattus* on bare reef limestone. *C. miliaris, C. lividus* and *C. flavidus* are much more generalized in their use of substrate types, and the other species shown are intermediate between the specialist and generalist categories.

Frequency
0 0,1 0,2 0,3 0,4 0,5 0,6 0,7 0,8 0,9
litteratus 139 leopardus 108 coronatus 426 abbreviatus 166 pennaceus 161 miliaris 239 lividus 468 flavidus 251 sponsalis 504 chaldaeus 270 ebraeus 787 rattus 265
bare limestone bench
algal turf on limestone bench
thin sand on limestone bench
rubble
sand under coral rock
sand
Species and Sample Size
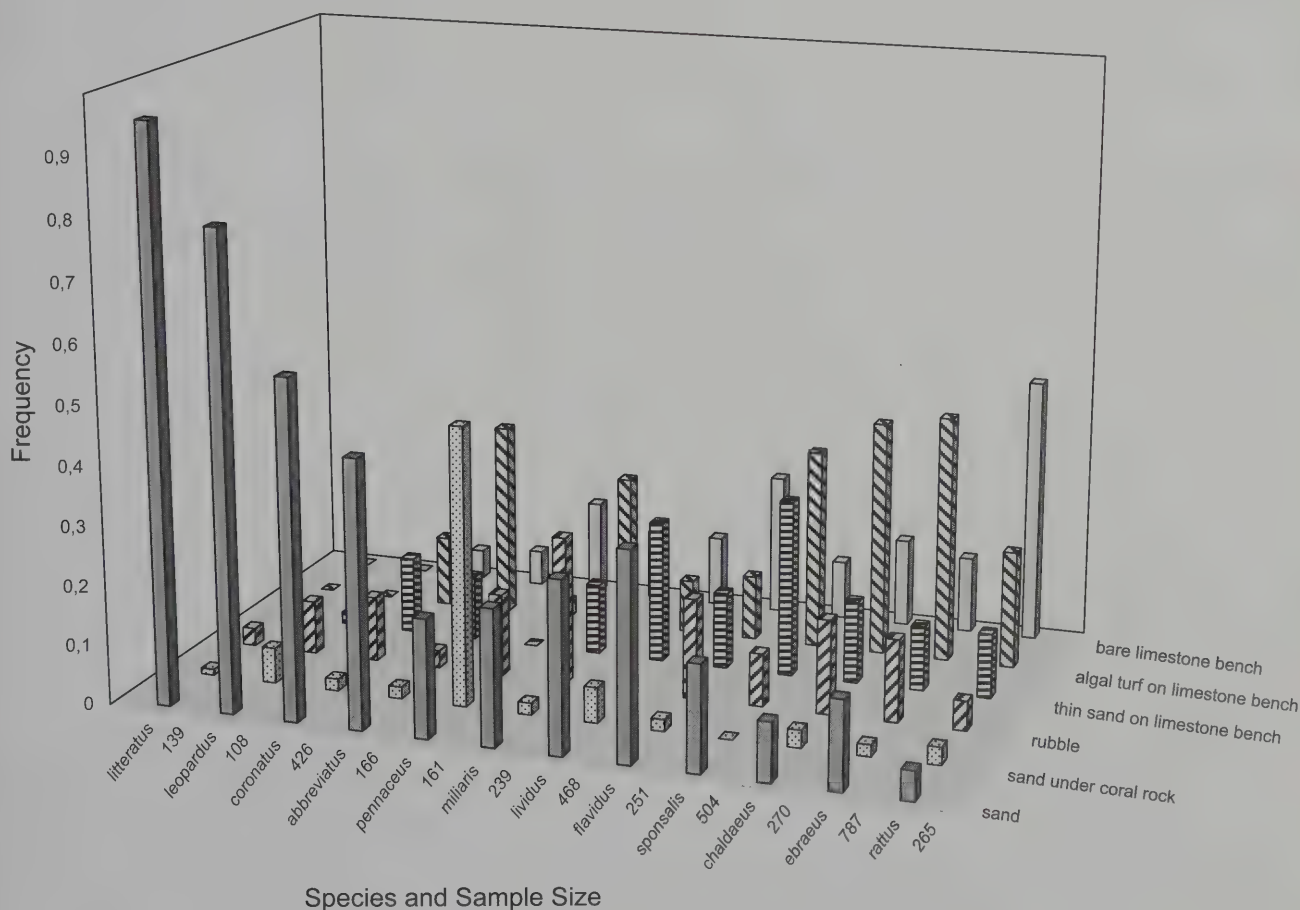
Fig. 4. Proportions of different substrate types used by 12 common *Conus* species in Indo-West Pacific coral reef-associated habitats. Sample sizes are to left of each species name. Bars represent the proportions of each substrate indicated at right used by each species. Species are arranged from those that occupy mainly soft substrates at left to those that occupy mainly hard substrates at right. Data are compiled from sources given in Table 1.

COMPARATIVE FEEDING BIOLOGY

Most *Conus* species prey exclusively or nearly so on members of only one major prey taxon: polychaete annelids, other gastropods, or fishes. Most species are in the first group, but a few of them also take unsegmented worms, particularly enteropneusts and echiurans. One species, *C. leopardus*, appears to prey only on the enteropneust *Ptychodera flava*. Within the predominant group of vermivorous species, co-occurring congeners have long been known to specialize on different taxa of polychaetes (KOHN, 1959, 1968; KOHN & NYBAKKEN, 1975). Table 1 summarizes the diets of the 12 commonest vermivorous species from Indo-West Pacific reef-associated habitats, and Fig. 5 shows the high degree of specialization at the prey family level of the 11 species with the largest samples of identified prey organisms (N>50).

Of the species shown in Fig. 5, some of the specialists on errant polychaetes prey more specifically on members of the family Eunicidae (*C. rattus, C. miliaris, C. miles*), one eats primarily nereids (*C. musicus*), and others eat both in similar proportions (*C. sponsalis, C. ebraeus*). Not shown because of smaller sample sizes are specialist predators of Amphinomidae (*C. imperialis, C. zonatus*). The commonest specialists on sedentary polychaetes are typically those *Conus* species that occupy soft substrates (compare Figs. 4 and 5). They prey mainly on members of the family Terebellidae (*C. frigidus, C. lividus*) and Capitellidae (*C. frigidus, C. litteratus*). Only *C. lividus* eats a substantial number of enteropneusts, and *C. leopardus* (not shown because of smaller sample size) feeds exclusively on them.

COMPARATIVE BIOLOGY IN THE LIGHT OF PHYLOGENY

The first species-level phylogenetic hypothesis of *Conus*, based on cladistic analysis of nucleotide sequences of the mitochondrial 16S rRNA gene and an intron located in a nuclear calmodulin locus, is now available for about 70 species (DUDA & PALUMBI, 1999; DUDA, KOHN & PALUMBI, 2001). The latter paper provides a tree based on both genes that is less well resolved than that for either alone but includes 13 clades comprising more than one (2-9) species, onto which feeding groups, based on Fig. 5, Table 1, and data for smaller samples, were mapped. These results may be summarized as follows:

In seven clades the *Conus* species whose diets are known feed primarily on errant polychaetes (designated as "E" clades). Five of these comprise specialists on eunicids: E1: (*C. coronatus*, ((*C. miliaris, C. abbreviatus*) (*C. chaldaeus* (*C. ebraeus, C. dorreensis*))));
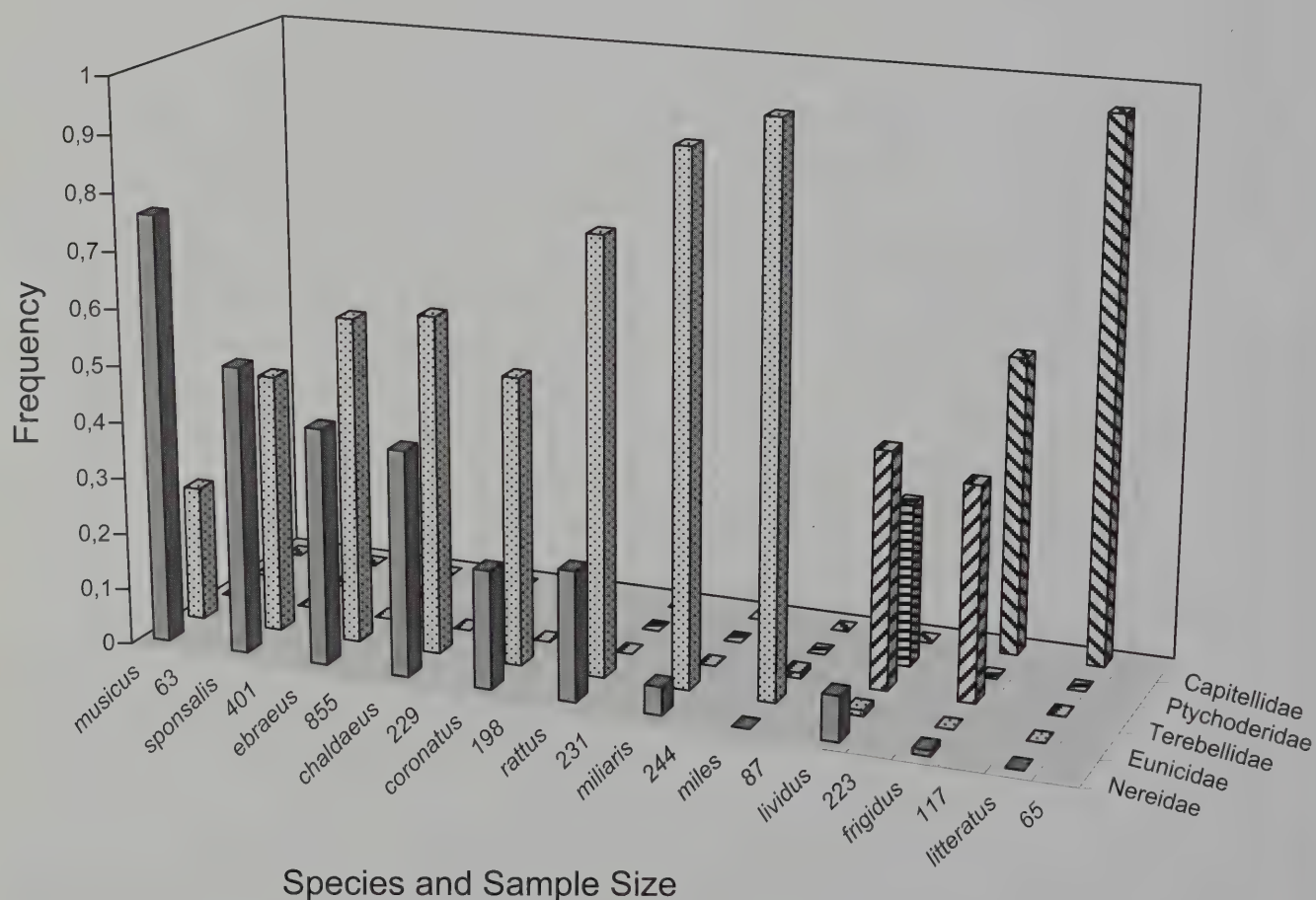
Fig. 5. Proportions of the most important prey families used by 11 common vermivorous *Conus* species in Indo-West Pacific coral reef-associated habitats. Sample sizes are to left of each species name. Bars represent the proportions of each prey family indicated at right consumed by each species. Species are arranged from those that prey mainly on errant polychaetes at left to those that prey mainly on sedentary polychaetes and a hemichordate at right. Data are compiled from sources given in Table 1.

E3: *C. eburneus*; E4: *C. biliosus*, (*C. balteatus, C. anemone*) *C. princeps*, and *C. glans*; E5: *C. vitulinus*; E6: *C. vexillum, C. rattus* (*C. mustelinus, C. miles*). Clade E2 comprises specialists on nereids: (*C. musicus* (*C. nux, C. sponsalis*)), and Clade E7, specialists on amphinomids: (*C. imperialis* (*C. regius, C. brunneus*)).

One clade (S1) comprises species that specialize on sedentary polychaetes of the family Terebellidae (*C. virgo* (*C. terebra* (*C. moreleti* (*C. flavidus, C. emaciatus*)))). Clade S2, (*C. quercinus* (*C. sanguinolentus, C. lividus*) includes species that prey, respectively, on several polychaete families and *Ptychodera*, Capitellidae, and Terebellidae and *Ptychodera*.

Three clades comprise species that prey on fishes, F1: (*C. stercusmuscarum* (*C. striatus*, (*C. catus* (*C. striolatus* (*C. magus, C. consors*))))); F2: (*C. obscurus* (*C. geographus, C. tulipa*)); and F3: (*C. proximus* (*C. circumcisus, C. cinereus*)). Finally, all of the molluscivorous species tested comprise one clade (M1): (*C. marmoreus, C. araneosus* (*C. episcopus, C. pennaceus, C. omaria* (*C. textile, C. canonicus*) (*C. legatus, C. aureus*)))). Molluscivorous *Conus* all prey exclusively on other gastropods, primarily other prosobranchs, but as in the case of the vermivores, they tend to specialize on members of different prey families (e.g. KOHN & NYBAKKEN, 1975). The diets of the four molluscivorous species with the largest identified prey samples (N=12-79), overlapped by only 0-29% (mean=10%) with respect to prey families.

In order to quantify the relation of similarity of prey taxa to phylogenetic affinity, a sample of 28 species was used, including those represented by smaller sample sizes (N=10-49) than those shown in Table 1 and Fig. 5. The euclidean distances of all pairwise comparisons of the proportions of each prey family in the diet were determined in a proximity matrix. Clade members are much more similar to each other with respect to the proportions of each prey family in the diet (mean distance =0.49; median=0.48) than are non-clade members (mean distance = median distance=0.90) (U test: z=5.2, P<0.0001). Species in the same clade are thus significantly more likely to have similar dietary requirements or preferences at the family level than are species in different clades.

As in the analysis of prey taxa used, similarity of microhabitat use was related to phylogenetic affinity. The sample comprised 36 species, including those represented by smaller sample sizes (N=10-64) and thus not shown in Fig. 5. The euclidean distances of all pairwise comparisons were again determined in a proximity matrix, and the values of clade members (mean=0.55; median=0.47) and non-clade members (mean=0.79; median=0.75) differed at the 0.0001 level (U test: z=5.1). Species in the same clade are thus significantly more likely to occupy similar substrates than are species in different clades. For example, four of the six species in clade E1 primarily utilize algal turf on limestone benches, both members of Clade E3 occur on broad expanses of sand, and five of the nine members of Clade M1 and three of the six in Clade F1 occur mainly on sand under coral boulders.

Finally, I reconsider the relationship of developmental mode,

Tab. 1 - Prey of the 12 commonest Indo-Pacific reef-associated vermivorous species of *Conus*. Numbers in body of table represent numbers of prey species at left recovered from guts of the predator species of *Conus* at top. The predominant prey item of each species is indicated by boldface, and the second most important, by italics. Sources: KOHN (1959, 1968, 1981, in press), KOHN & ALMASI, 1993; KOHN & NYBAKKEN (1975), LEVITEN (1976), and unpublished data.

Species *Conus*	*sponsalis*	*ebraeus*	*chaldaeus*	*rattus*	*distans*	*vexillum*	*miliaris*	*coronatus*	*miles*	*frigidus*	*flavidus*	*lividus*
Nereidae												
Nereis jacksoni	116	93	6					4				
Perinereis singaporiensis	67	*272*		21								
Platynereis dumerilii	26		154	1				1				16
Eunicidae												
Lumbrineris sarsi	38											
Palola siciliensis	1	344	*92*					4	45			
Eunice afra	*83*	6		101	48		34	18				1
Eunice antennata	3			*31*	2	20		5	1			
Lysidice collaris	41	8		1			64	30	*22*			
Nematonereis unicornis	5	9					*42*	23	1			
Marphysa sanguinea												
Glyceridae												
Glycera tesselata	7					*10*		30				
Capitellidae												
Dasybranchus caducus	1							*27*		62	*25*	
Terebellidae												
Loimia medusa									1	*23*		*40*
Polycirrus medius										9	22	20
Thelepus setosus										2	11	18
Nicolea gracilibranchia											36	15
Maldanidae												
Axiothella australis										7	1	30
Hemichordata												
Ptychodera flava											5	65

particularly the loss of a planktonic larva, in *Conus* to phylogenetic affinity. DUDA & PALUMBI (1999) mapped this trait on their initial phylogenetic tree, based only on the calmodulin intron sequence, and concluded that all eight species on the tree that lack a planktonic stage in the life history evolved this trait independently from ancestors with planktonic larvae. Optimizing this trait to the combined 16S rRNA and calcomdulin tree (DUDA, KOHN & PALUMBI, 2001) indicates a different pattern. The seven species with sequence data for both genes now belong to four clades, equivalent to E4 (*C. anemone, C. boeticus*), F1 (*C. magus*), F3 (*C. cinereus, C. proximus*) , and M1 (*C. araneosus, C. pennaceus*). Thus while the planktonic larva still appears to have been lost on several occasions during the evolution of *Conus*, all such cases may not have been independent. The small sample size (N=8) makes statistical analysis difficult. However, the distribution of species with non-planktonic development among clades does not differ significantly from the expected Poisson distribution (x^2=4.8; 0.1<P<0.05).

DISCUSSION AND CONCLUSIONS

Conus, with more than 500 extant species the largest genus of marine molluscs, is also characterized by very large numbers of co-occurring species, particularly in coral reef-associated habitats of the Indo-Pacific region. For the past half century, studies of comparative biology of these assemblages have provided insights into geographic patterns of species diversity, use of food and substrate resources, life history and its relationship to biogeography, and patterns of evolutionary diversification. In all prior studies, for statistical purposes each *Conus* species had to be treated as an independent entity. This is clearly an oversimplification that is likely to lead to erroneous interpretations, because surely some species are more and others less closely related phylogenetically to each other. And similarity between species with respect to patterns of the attributes listed above results from intrinsic genetic or genealogic history as well as from adaptive responses to extrinsic ecological factors. However, it was not possible to incorporate this information into prior comparative studies. The reason of course is that although systematists since Linnaeus had hypothesized the existence of groups of related species within *Conus*, no phylogeny had ever been proposed. By providing the first objective, species-level phylogenetic hypothesis, the recent molecular phylogenetic study of more than 70 mainly Indo-Pacific species by Thomas Duda is an important advance toward removing this long-standing roadblock to understanding the comparative biology of *Conus*. Data sets on habitat and food resource use are shown to have a strong phylogenetic signal. That is, species within clades identified in the molecular study use significantly more similar substrate and prey types than do species that belong to different clades. This alone provides strong evidence that species are not independent entities for statistical purposes, and they should not be so treated. Future studies that address the problem of non-independence quantitatively, using methods of independent comparisons (e.g. FELSENSTEIN, 1985; PAGEL, 1992), as well as extending sequence analyses to additional genes, species, and populations will lead to a much fuller understanding of comparative biology of the most diverse genus in the sea.

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Feeding habits of *Chicoreus capucinus* (Neogastropoda: Muricidae) in a Singapore mangrove

Koh-Siang Tan & Tre Ming Oh

KEY WORDS: Indo-Pacific, mangrove, ecology, feeding habit, Mollusca, Crustacea.

ABSTRACT Molluscs form a substantial component of the resident fauna in tropical mangroves. While the majority of them are either herbivorous gastropods or filter-feeding bivalves, predatory gastropods can be common and may therefore exert considerable influence on mangrove biotic structure. One well-known, common predator in Indo-Pacific mangroves is the muricid gastropod *Chicoreus capucinus*. Despite its large size and common occurrence, its biology and feeding habits are poorly known, when compared to its rapanine counterparts. In this study, detailed field observations were made in a disturbed mangrove fringing the West Johor Straits in Singapore. A total of 15 bivalve species, 7 gastropod species and 2 crustacean species were recorded from 341 occurrences of predation in the field. Bivalves constituted 83% of total prey consumed, most of which were drilled. Infaunal lantern shells (*Laternula* cf. *boschasina, L. truncata*), and epifaunal mussels (*Modiolus* cf. *metcalfei*) comprised 37% and 22% of prey drilled, respectively. Gastropods drilled and preyed upon were mainly small, consisting of rissooidean and cerithioidean species. The main crustacean prey was the barnacle *Balanus amphitrite*, although the wood-boring isopod *Sphaeroma* sp. was also consumed. Analysis of gut contents did not reveal additional prey species. The results show that *C. capucinus* is a versatile predator of molluscs and crustaceans, seeking, attacking and consuming a wide variety of prey from different components of the mangrove habitat.

RIASSUNTO I molluschi sono una componente molto importante della fauna residente nelle comunità tropicali delle mangrovie. Per la maggior parte si tratta di gasteropodi erbivori o bivalvi filtratori; ciononostante gasteropodi predatori sono frequentemente comuni e possono in tal caso avere una influenza considerevole sulla struttura biotica del mangrovieto. Un predatore ben noto e realtivamente comune in Indo-Pacifico è *Chicoreus capucinus* (Gastropoda, Muricidae). Nonostante le grandi dimensioni e la relativa frequenza, la sua biologia e le abitudini alimentari sono poco note, soprattutto se in confronto con le controparti Rapaninae. In questo studio sono riportate le osservazioni dettagliate sul campo in mangrovie disturbate nel West Johor Straits di Singapore. Un totale in specie di 15 bivalvi, 7 gasteropodi e 2 crostacei sono stati rinvenuti in 341 casi di predazione nel campo. I bivalvi, per la maggior parte perforati, costituiscono l'83% del totale delle prede consumate. I Laternulidae dell'infauna (*Laternula* cf. *boschasina*, *L. truncata*), e i mitilidi epifaunali (*Modiolus* cf. *metcalfei*) rapprenentano rispettivamente il 37% e il 22% delle prede perforate. I gasteropodi perforati e predati erano principalmente di piccola taglia, con specie di rissooidei e cerithioidei. I principali crostacei erano balani (*Balanus amphitrite*), benché anche l'isopode xilofago *Sphaeroma* sp. fosse presente nella dieta. L'Analisi del contenuto stomacale non ha rivelato ulteriori specie tra le prede. I risultati mostrano che *C. capucinus* è un predatore versatile di molluschi e crostacei, che ricerca, attacca e consuma un'ampia varietà di prede tra i differenti componenti dell'habitat delle mangrovie.

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INTRODUCTION

Chicoreus capucinus (Lamarck, 1822) is a relatively large (up to 125 mm shell height but typically up to 50 mm) intertidal muricine neogastropod that is common and often abundant in mangroves of Southeast Asia (BERRY, 1963; BRANDT, 1974; SASEKUMAR, 1974; FRITH, TANTANASIRIWONG & BHATIA, 1976; WONG, CHARLES & KHOO, 1984; HOUART, 1992; WONG & AHMAD, 1996; MIDDLEFART, 1997). In Singapore, *C. capucinus* is found in pockets of highly disturbed mangroves along the East and West Johor Straits, a narrow estuarine channel separating Singapore Island from peninsular Malaysia. The species occupies a broad zone covering the landward edge to the seaward fringe (BERRY, 1972) and occurs in a wide variety of habitats ranging from mangrove tree trunks (MORTON, 1976a as *C. adustus*), algal mats to muddy sandbanks, as well as artificial substrates such as jetty pilings and monsoon drain walls. Its common occurrence in the mangroves, however, belies our knowledge of the biological role in this increasingly threatened tropical ecosystem. Several studies have examined its basic biology at Phuket Island in Thailand (e.g., NIELSEN, 1976; MIDDLEFART, 1996; AUNGTONYA & VONGPANICH, 1997; GRIBSHOLT, 1997) and development of imposex (SWENNEN ET AL., 1996; TAN, 1999) but, in general, molluscan predator-prey relationships in the mangroves are poorly understood. *Chicoreus capucinus* is exceptional amongst members of the Muricinae because of its estuarine distribution. At the same time, it is also one of a few gastropod predators present in substantial numbers in the mangal. This study aims to investigate, through field and laboratory observations, the feeding biology of *C. capucinus* in the mangroves of Singapore. The natural diet was determined by examining the stomach and rectal contents of individuals collected from the mangroves. Direct observations in the field and feeding experiments in aquaria were also carried out to determine feeding techniques.

MATERIALS AND METHODS

Field observations

Field observations were made over a period of five weeks, during September-October 1999 and again in February 2000, in a small area of disturbed mangrove (1° 26.8'N, 103° 44.1'E) near Sungei Buloh Nature Park on the northwest coast of Singapore fronting the West Johor Strait. An area about 1.5 ha in size adjacent to the Nature Park was selected as the study site. Each visit lasted about three hours in daylight at low tide, during

which the mangrove floor was searched thoroughly for *Chicoreus capucinus*. A total of ten visits was made. For each individual found in the process of attacking/consuming prey, the size and identity of the item were recorded as was the size of the predator. Density of individuals were determined by randomly throwing 50cm x 50cm quadrats in the area sampled. Location and size of drill holes on individual prey were determined in the laboratory. Similar observations were also made on *C. capucinus* preying upon barnacles on mangrove tree trunks.

Laboratory and aquarium observations

Chicoreus capucinus individuals from the same locality were dissected and examined for stomach and rectal contents. They were cracked and fixed immediately in 4% seawater formaldehyde after collection. After fixing for 48 hours, they were washed in tap water and stored in 80% ethanol. Gut contents were removed and mounted on slides in Aquamount (TAYLOR, 1984). These were examined under the microscope and compared with intact prey specimens obtained from the same habitat. To observe and document feeding in aquaria, 20 *C. capucinus* were starved for one month, after which they were kept singly in perforated vials submerged in aerated seawater and prey introduced in turn to them. The following two gastropods and six bivalves were provided as prey items: *Littoraria vespacea* (Littorinidae), *Xenostrobus* cf. *atratus* (Mytilidae), *Brachidontes* sp. (Mytilidae), *Perna viridis* (Mytilidae), *Isognomon ephippium* (Isognomonidae), *Saccostrea cuccullata* (Ostreidae) and *Enigmonia aenigmatica* (Anomiidae). All of these occur in the same habitat as *C. capucinus*. The prey items in the vials were examined carefully for evidence of predation after five days.

RESULTS

Of a total of 2097 *Chicoreus capucinus* individuals located on the mangrove floor, 341 (16.2%) were found in association with prey. In the majority of cases, each predator was associated with a single prey item, and prey sharing was observed on only a few occasions. Individuals were well spaced apart, with a mean density of 0.9±1.0 individuals per 2500 cm^2 (n=200 quadrats; range between 0 and 5 individuals). The size of *C. capucinus* with prey ranged between 10.8 and 51.7 mm in shell height. A total of 24 prey species was identified, comprising 15 bivalve species, seven gastropod species and two species of crustaceans (Table 1). Bivalve prey items ranged from juvenile mussels 1.4 mm in shell length to adult *Laternula truncata* with a shell length of 44.3 mm. Gastropod prey ranged between 2.8 mm in shell height (*Iravadia* sp.) to 18.4 mm (juvenile *Cerithidea obtusa*). Bivalves constituted 83% of the total prey items identified. These included the epifaunal mussels *Modiolus* cf. *metcalfei* Hanley and *Brachidontes* sp. which are byssally attached to and found amongst strands of the densely matted *Chaetomorpha gracilis*, as well as the infaunal bivalves green alga *Diplodonta* sp., *Dosinia* sp., *Marcia marmorata*, *Geloina erosa, Tellina* sp., *Glauconome virens, Laternula* cf. *boschasina* and *L. truncata.* Gastropods comprised less than 10% of the total prey consumed, and most of these were either *Iravadia* sp. (12 observations) or *Cerithidea obtusa* (8 observations). Other gastropods eaten included juvenile *Nerita*, *Telescopium,* onchidiids and *Salinator.* The remaining 7% were two crustaceans: the barnacle *Balanus amphitrite amphitrite* (29 observations) and the wood-boring isopod *Sphaeroma* sp. (5 observations).

Gut-contents analysis of 44 specimens of *Chicoreus capucinus* from the mangroves revealed that 29.5% of predators had barnacle exoskeletons (*Balanus amphitrite*) while 31.8% contained unidentifiable soft tissue.

In the aquarium, juvenile neritid (*Nerita* sp.) and adult littorinid gastropods (*Littoraria vespacea*) were preyed upon and eaten. *Nerita* sp. was mostly drilled either through the shell (50.0% of the cases) or at the edge of the calcareous operculum (33.3% of cases). The rest were consumed without any evidence of drilling. For the littorinid *L. vespacea*, about half were drilled through the edge of the operculum, whilst the remainder were either drilled through the shell at the suture between the last and penultimate whorls, or were attacked and consumed with no drill marks. Of the bivalves, *Xenostrobus* cf. *atratus*, *Brachidontes* sp., *Perna viridis*, *Isognomon ephippium*, *Saccostrea cuccullata* and *Enigmonia aenigmatica* supplied to the predators, three (*Brachidontes, Saccostrea* and *Enigmonia*) were all drilled and eaten, while *Xenostrobus, Perna* and *Isognomon* were neither drilled nor consumed by *C. capucinus* during the course of the experiments (Table 2).

Most prey was attacked by boring. Drill holes were present in the majority of prey items (78.8% of total) observed in the mangroves. Both bivalve and gastropod prey were drilled. Typically, the drill hole was a circular perforation between 0.4 and 1.5 mm in diameter surrounded by an irregular corroded region. In general the diameter of the drill holes made correlated with predator size (r=0.365, P<0.01 [n=105] for drill holes on *Laternula* cf *boschasina*; r=0.692, P<0.01 [n=69] on *Modiolus* cf. *metcalfei*). *Chicoreus capucinus* overwhelmingly preferred to drill the posterior regions of both bivalves (Figure 1). Despite the infaunal habits of *Laternula* and its permanent shell gape, the drill holes were concentrated on the posterior half of either valve.

These observations also show that *C. capucinus* is capable of attacking and consuming prey by other, as yet undetermined, methods. The method of attack seems to differ for different prey. Whilst 79% and 89% of *Laternula* cf. *boschasina* and *Modiolus* cf. *metcalfei* were drilled, 52% of *Laternula truncata* and 44% of *Marcia marmorata* were consumed with no evidence of drilling by *C. capucinus* (Table 1). Nearly 90% of barnacles consumed by *C. capucinus* were not drilled. About 30% of Iravadia sp. and *Cerithidea obtusa* appear to have been attacked and consumed without drilling. One method employed by *C. capucinus* when feeding on gastropods is access via the operculum. *Littoraria vespacea* was drilled through their operculum in many cases (see above).

The anomalodesmatan bivalve *Laternula* cf. *boschasina* and an epifaunal mussel *Modiolus* cf. *metcalfei* were the two major prey items of *C. capucinus* on the mangrove floor. They comprised 37.1% and 21.5% of the total prey items eaten, accounting for nearly half of the predatory activities of *C. capucinus*. There was,

however, a significant difference in the mean size of *C. capucinus* feeding on *L.* cf *boschasina* and those feeding on *Modiolus* cf. *metcalfei* (t-statistic=14.11, df=105, P<0.001). Those feeding on *Laternula* were significantly larger than those preying on *Modiolus*. The mean shell height of *C. capucinus* individuals preying upon *L.* cf *boschasina* was 37.7±5.1mm (n=126), while the mean size of those preying upon *Modiolus* was 22.7±8.2mm (n=73). The average shell length of *L.* cf *boschasina* was 16.7±4.0mm

Table 1. Bivalves, gastropods and crustaceans preyed upon by *Chicoreus capucinus* on the mangrove floor at Sungei Buloh Nature Park, West Johor Strait, Singapore based on field observations.

Prey items	Total no. of observations	% drilled	Prey size range (mm)	Predator size range (mm)
Bivalves				
Barbatia sp. (Arcidae)	2	100.0	9.2 and 12.5	33.5 and 51.0
Brachidontes sp. (Mytilidae)	5	100.0	6.3–13.5	15.5–29.4
Modiolus cf. *metcalfei Hanley* (Mytilidae)	73	89.0	1.4–12.4	10.8–39.9
Musculista senhousia (Benson in Cantor) (Mytilidae)	21	100.0	4.4–14.7	13.9–39.1
Undet. Mytilidae	1	100	15.9	42.4
Diplodonta sp. (Ungulinidae)	6	66.7	6.4–21.3	33.7–51.7
Marcia sp. (Veneridae)	9	55.6	5.3–25.0	19.2–39.1
Dosinia sp. (Veneridae)	1	100	11.8	44.4
Undet. Veneridae	2	0	5.3 and 5.6	26.2 and 42.2
Geloina erosa (Solander) (Corbiculidae)	7	100.0	4.9–15.3	24.7–39.1
Undet. Tellinidae	4	25.0	11.9–29.2	30.0–35.7
Glauconome virens (L.) (Glauconomidae)	1	100	14.0	33.9
Laternula cf. *boschasina* (Valenciennes in Reeve) (Laternulidae)	126	79.4	7.8–30.4	26.4–49.3
Laternula truncata (Lamarck) (Laternulidae)	21	47.6	8.9–44.3	27.7–48.3
Martesia striata (L.) (Pholadidae)	2	100.0	not measured	not measured
Gastropods				
Nerita sp. (Neritidae)	2	100.0	not measured	not measured
Iravadia sp. (Iravadiidae)	12	66.7	2.8–4.1	9.5–16.9
Fairbankia sp. (Iravadiidae)	1	0	3.3	17.7
Cerithidea obtusa (Lamarck) (Potamididae)	8	62.5	6.9–18.4	32.2–37.7
Telescopium telescopium (L.) (Potamididae)	1	100	11.0	40.5
Onchidium sp. (Onchidiidae)	1	0	6.1	28.3
Salinator cf. *burmana* (Blanford) (Amphibolidae)	1	100	6.3	40.4
Crustaceans				
Balanus amphitrite Darwin (Cirripedia)	29	20	not measured	not measured
Sphaeroma sp. (Isopoda)	5	0	not measured	not measured

Table 2. Gastropod and bivalve prey items consumed by *Chicoreus capucinus* in perforated vials submerged in aquaria. All prey items are found in the mangroves. Each vial contained one predator and one prey item (n=20). The prey were examined after five days. (*) denotes drill holes through the shell or the operculum.

Molluscan prey item presented to *Chicoreus capucinus*	No of prey presented	No of prey eaten after five days	No of prey drilled	No of prey eaten without drill holes
Nerita sp. (Neritidae)	20	12	10	2
Littoraria vespacea Reid (Littorinidae)	20	20	14*	6
Xenostrobus cf. *atratus* (Lischke) (Mytilidae)	20	0	0	0
Brachidontes sp. (Mytilidae)	20	20	20	0
Perna viridis (L.) (Mytilidae)	20	0	0	0
Isognomon ephippium (L.) (Isognomonidae)	20	0	0	0
Saccostrea cuccullata (Born) (Ostreidae)	20	20	20	0
Enigmonia aenigmatica (Holten) (Anomiidae)	20	20	20	0
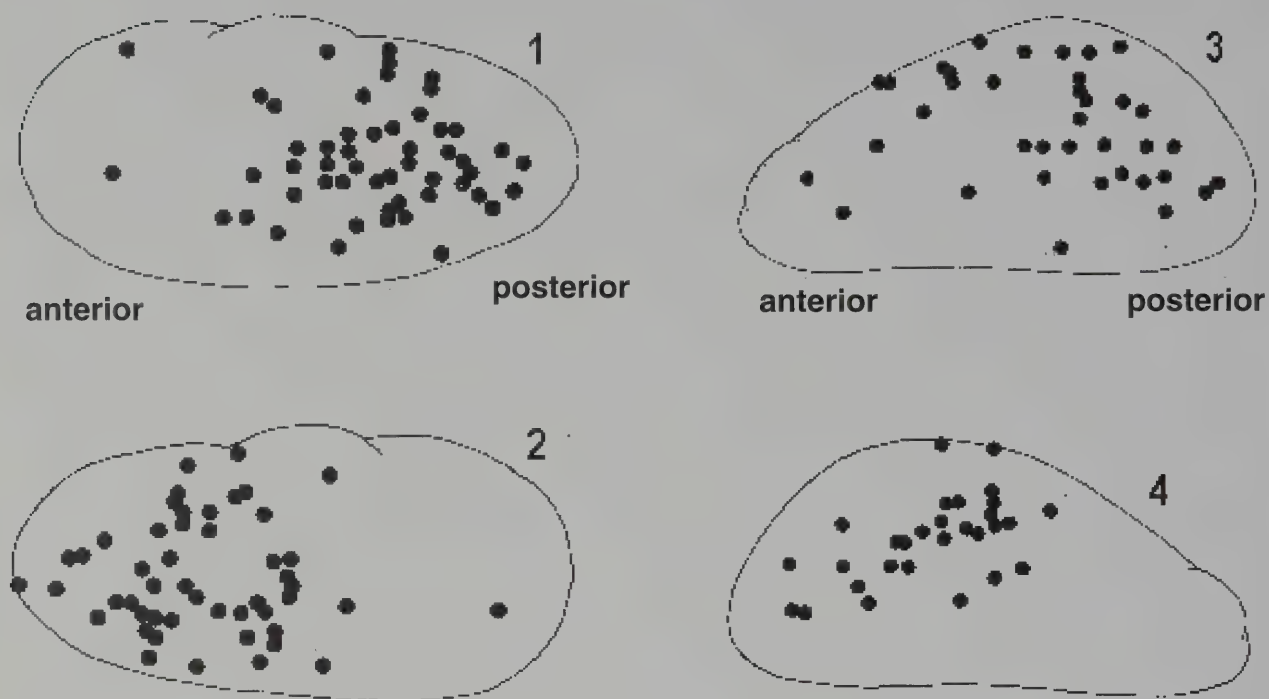
Figure 1. Location of drill holes on left (top row) and right (bottom row) shells of (1, 2) *Laternula* cf. *boschasina* and (3, 4) *Modiolus* cf. *metcalfei* from a total of 199 observations of predation on the two bivalve species by *Chicoreus capucinus* at Sungei Buloh mangroves. In *Laternula,* a total of 52 left valves and 48 right valves were drilled. In the case of *Modiolus,* a total of 36 left valves and 29 right valves were drilled. In all cases, one drill hole was associated with each valve

and that of *Modiolus* was 6.7±2.3mm. Size correlation between predator and prey size was evident within each prey type. A weak but significant relationship ($r=0.196$, $P<0.05$, $n=126$) was detected for *C. capucinus* preying upon *Laternula,* while a stronger significant correlation was determined between *C. capucinus* and *Modiolus* ($r=0.357$, $P<0.01$, $n=73$). Significant positive correlation was observed between mean sizes of predator and prey ($r=0.694$, $P<0.01$, $n=11$) (Figure 2), i.e., predators generally preyed upon bivalves and gastropods that were in proportion to their size on the mangrove floor.

On mangrove tree trunks, the predominant prey was *Balanus amphitrite.* Again, prey-sharing was not observed, although the abundance of prey on the trees may account for the high densities of *C. capucinus* on barnacle colonies. Of a total of 200 observations, 76 individuals (38%) were seen attacking or feeding on barnacles. The mean size of *C. capucinus* preying upon these was 32.4±5.4mm (n=76) and the mean barnacle size (apertural length) was 2.9±0.9mm (n=76). Only eight of 76 barnacles being attacked/consumed had drill holes on their opercular plates. No drill holes were present on the external wall plates. The majority of barnacles attacked did not show any sign of drilling and, presumably, *C. capucinus* had forced its proboscis between the plates to gain access to the animal beneath.

DISCUSSION

The diets of a number of rocky-shore and coral reef rapanine gastropod species are well investigated. Several species of *Morula* and *Thais* feed on barnacles, polychaetes, sipunculans, chitons, various bivalves as well as other gastropods (TAYLOR, 1978, 1980, 1984; ABE, 1980, 1989; TONG, 1986; HARPER & MORTON, 1997; TAYLOR & GLOVER, 1999). By comparison, data on muricine gastropods are in general lacking (GRAHAM, 1955; RADWIN & D'ATTILIO, 1976; TAYLOR, 1982; PONDER & VOKES, 1988). WELLS (1958) investigated the diet of *Murex fulvescens* Sowerby which comprised oysters, mussels, scallops and tellinids. In Florida, *M. pomum* Gmelin drilled oysters (MENZEL & NICHY, 1958) and *M. florifer* Reeve fed predominantly on the venerid bivalve *Chione* (PAINE, 1963). In Hong Kong, polychaetes, crustaceans, fish remains and unidentified tissue, possibly of molluscan origin, were found in the gut of *M. trapa* Röding, indicating their capacity to feed on a variety of prey and carrion (TAYLOR, 1982). For what little is known about the diet of *Chicoreus* species, most are able to feed on bivalves. Chicoreus *ramosus* (L.) fed on *Anadara granosa, Perna viridis, Modiolus metcalfei, Meretrix meretrix* and *Ruditapes* sp. in aquaria, and were seen to feed on the giant clam *Tridacna crocea* in the field (RUANGCHOY & TANTICHODOK, 1991). Both *C. ramosus* and *C. virgineus* (Röding) can drill the venerid bivalve *Meretrix meretrix*

(Patterson Edward, Raghunanthan & Ayyakkannu, 1992). Taylor (1980) observed that *C. brunneus* and *C. micropyllus* fed principally on bivalves (mostly *Alectryonella plicatula*) and barnacles (*Balanus trigonus*) in Hong Kong. In Singapore, *C. torrefactus* (Sowerby) drills *Gafrarium pectinatum,* another venerid bivalve (pers. obs.). Nielsen (1976) reported that *C. capucinus* fed on *Saccostrea cucullata* and *Balanus amphitrite.* Gribsholt (1997) observed *C. capucinus* feeding mainly on *S. cucullata,* but other molluscs were also eaten, including *Cultellus* sp., *Cerithidea cingulata* (laboratory observations), and possibly shipworms (*Teredo* sp.). In contrast to what amounts to a largely bivalve diet, the potamidid gastropod *Cerithidea* was the main prey of *C. capucinus* at Ang-Sila and Kungkraeban Bay in the Gulf of Thailand, although other gastropods and bivalves were consumed as well (Wells, Chalermwat, Kakkai & Rangubpit, unpublished observations). These studies indicate that it is capable of feeding on a variety of prey, but at the same time they suggest that its dietary composition varies according to the predominant prey available at a particular location. In the Sungei Buloh mangroves, bivalves constitute the main prey type of *C. capucinus.* It is at home both on hard and soft substrata (unlike its counterpart *Thais gradata,* which is confined to mangrove tree trunks and prop roots), and a tolerance for a wide range of salinities as well as an ability to withstand dessication (pers. obs.) are likely factors that allow access to a wide range of prey.

Field observations showed that juveniles may have different diets compared to adults. *Chicoreus capucinus* feeds on a variety of gastropods and bivalves, and possibly switches from a diet of small gastropods and mussels to larger gastropods, barnacles and other bivalves as it grows. Such size-related prey selection and associated versatility in prey handling were also seen in *Thais clavigera* (Küster) and *T. luteostoma* (Holten), two common intertidal muricids in Hong Kong (Taylor & Morton, 1996). While positive prey-predator size relationships have been demonstrated for several other species of Muricidae (e.g., Palmer, 1988; Taylor, 1990) such relationships are by no means the norm for neogastropods. In other studies, there was no clear relationship between predator size and prey size (e.g., Tan & Morton, 1998; Taylor & Glover, 1999). Further studies are in hand to determine the relative importance of prey availability, accessibility and energy value in determining the diets of juvenile and adult neogastropod predators. Nevertheless, it is clear from the limited aquarium observations that a wide variety of potential prey items of various sizes can be exploited by *C. capucinus.* Littorinids such as *Littoraria vespacea,* and the anomiid bivalve *Enigmonia aenigmatica* are usually out of reach of most *C. capucinus* in the field as they occupy non-overlapping niches, but they are readily drilled and eaten when circumstances allow.
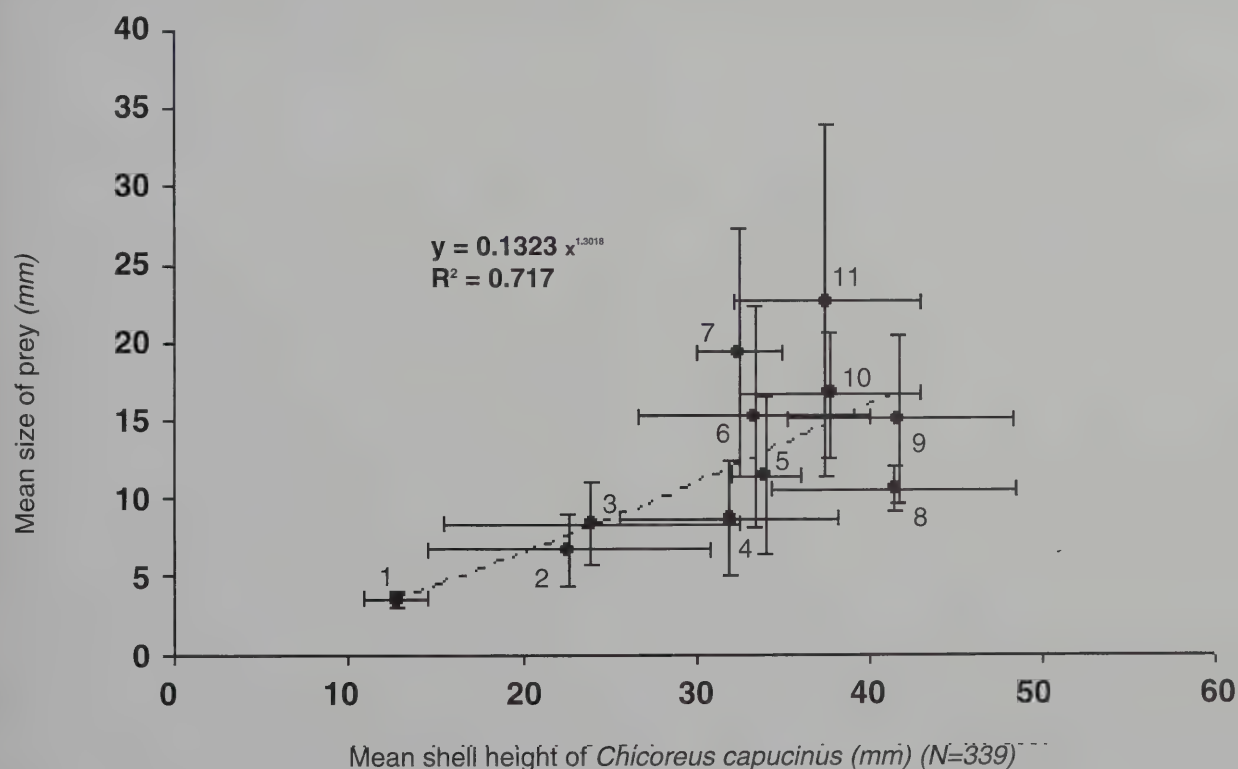
Figure 2. Predator-prey size relationship between *Chicoreus capucinus* and its molluscan prey items at Sungei Buloh mangroves. The mean shell height and standard deviation (±SD) of *C. capucinus* individuals seen feeding upon a particular prey type (nos. 1–11) and the mean size (±SD) of prey are plotted for each of the 11 gastropod and bivalve prey. Prey types: 1–*Iravadia* sp.(n=12); 2–*Modiolus* cf. *metcalfei* (n=73); 3–*Musculista senhousia* (n=21); 4–*Geloina erosa* (n=7); 5–*Cerithidea obtusa* (n=8); 6–*Marcia* sp. (n=9); 7–*Tellina* sp. (n=4); 8–*Barbatia* sp. (n=6); 9–*Diplodonta* sp. (n=6); 10–*Laternula* cf. *boschasina* (n=126); 11–*Laternula truncata* (n=21). Sample sizes with less than four prey items were excluded.

Plate 1. *Chicoreus capucinus* feeding on *Laternula* cf. *boschasina* on the mangrove floor at Sungei Buloh Nature Park, Singapore. The predator was lifted away from the substrate to reveal the prey under its foot in this photograph.

Drilling is the predominant technique used to gain access to prey with external shells on the mangrove floor. The absence of accessory salivary glands in *C. capucinus* (pers. obs.) may be one reason why drilling is used extensively for feeding. Other predatory gastropods such as *Morula* and *Thais* with prominent accessory salivary glands can attack and consume bivalves without leaving drill marks (TAYLOR & MORTON, 1996; pers. obs.). Secretions of the accessory salivary glands and the hypobranchial gland have been found to contain choline esters that may be used to paralyse prey (ANDREWS, ELPHICK & THORNDYKE, 1991). At least the former is technically unavailable to *C. capucinus,* and careful observations on both drilled and undrilled prey have not revealed evidence of coloured hypobranchial secretions on them, although the prey is usually smothered by clear mucus. It is not immediately known if such secretions originate from prey or predator. How *C. capucinus* attacks and consumes prey without drilling is therefore unclear. In both field and aquarium observations, neritid gastropods were sometimes consumed without drill marks on either the shell or its operculum. This implies that *C. capucinus* was somehow able to insert its proboscis between the operculum and the apertural wall of a *Nerita* shell. It is unclear how this is achieved. *Chicoreus capucinus* might have gained access by smothering (and eventual asphyxiation of prey) or may have used toxic secretions from its hypobranchial gland, causing paralysis of prey, as suggested for *Dicathais orbita* preying on the turbinid *Ninella torquata* which has a thick, calcified operculum (Taylor & Glover, 1999). Similarly, no sign of drilling was evident on either the wall or opercular plates of most barnacles attacked by *C. capucinus*, in contrast to other muricids (HART & PALMER, 1987).

In the case of bivalves, *C. capucinus* can also attack and consume them without resorting to drilling. Again, the exact mechanism remains unknown, although similar methods used for overcoming gastropods and barnacles are likely to be used here. However, WELLS (1958) found that *Murex fulvescens* was capable of pulling the valves of oysters and other bivalves apart using its foot. Such a technique does not usually leave evidence of predation and more detailed observations are necessary.

The predominance of *Laternula* as a prey item of *C. capucinus* is interesting. The predators appear to prey on these lantern shells rather than, for example, the more abundant and conspicuous epibyssate bivalve *Isognomon ephippium,* as observed in the field and in aquaria. Though *Laternula* has a thin shell with a permanent gape and is generally immobile as adults

(MORTON, 1976b; SAVAZZI, 1990), the well camouflaged, sand-encrusted siphons, infaunal habits (they are usually buried at depths of 2 cm or more below the substratum, and are virtually impossible to detect visually on the mud surface) and possession of well-developed, complex siphonal eyes mounted on the tip of siphonal tentacles (ADAL & MORTON, 1973; MORTON, 1973; NILSSON, 1994) are predator defences. In particular, the shadow reflex described by MORTON (1973), i.e., the flicking of siphonal tentacles in response to a shadow, could flick sand grains over its siphons, possibly making it difficult for *C. capucinus* to grasp the siphons as they withdraw on contact, which are themselves likely to be "drill-proofed" by the layer of sand grains attached to them. Nonetheless, *C. capucinus* appears to have little difficulty in detecting, attacking and consuming both small and large *Laternula* individuals. From numerous observations it seems likely that *C. capucinus* extends the anterior region of its foot into the substratum and by wrapping its foot around the posterior region, pulls out *Laternula* from its burrow. It then proceeds to drill the shell on the substratum surface. Drill holes were made on the posterior half of the bivalve, also on the left or right valve (and infrequently at the hinge). The sand-encrusted, tough-skinned siphons were usually left uneaten.

The relatively high frequency of predation by *C. capucinus,* its catholic diet and common occurrence all suggest that this predator plays a significant role in the ecology of Sungei Buloh mangroves. It is capable of exploiting a wide range of prey and shows great versatility in handling both bivalves and gastropods from different substrata.

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Foregut ontogeny of the Neogastropoda: comparison of development in *Nucella lapillus* and *Conus anemone*

Alexander D. Ball

KEY WORDS: Gastropoda, Neogastropoda, Muricoidea, Conoidea, Development, Ontogeny.

ABSTRACT Characters of the foregut organs of neogastropods have been widely used in phylogenetic analyses. These include, especially, the two pairs of salivary glands, the radula, the valve and gland of Leiblein in muricids and the venom apparatus of conoideans. Assumptions and hypotheses concerning the homologies of these organs both within the Neogastropoda and with other gastropods can be tested by ontogenetic studies. Developmental stages of *Nucella lapillus* (Muricoidea) and *Conus anemone* (Conoidea) were studied through reconstruction of 1µm resin-embedded sections, light and scanning electron microscopy.

In both *Nucella* and *Conus* the buccal mass is derived from a ventral evagination of the oesophagus. An anterior portion forms the sub-lingual pouch in *Nucella* and the radular caecum in *Conus*, whilst the posterior portion forms the radular sac. The acinous salivary glands are derived as lateral evaginations of the wall of the buccal cavity. This region moves dorsally in *Nucella* and the salivary gland ducts grow posteriorly attached to the anterior oesophageal wall. In *Conus*, the buccal cavity is separated from the oesophagus by the buccal sac. The acinous salivary glands initially grow laterally from its walls and then dorsally as the secretory regions expand. They do not become associated with the oesophageal walls. The accessory salivary glands in both species arise as paired evaginations of the ventral lip, the ducts grow posteriorly and terminate in secretory areas. During the development of *Nucella* the ducts fuse, but terminate in paired, secretory regions. In *Conus*, the paired glands fuse completely during development, leaving a single tubular gland. The valve of Leiblein in *Nucella* is derived from the dorsal folds and dorsal wall of the mid-oesophagus. This region differentiates prior to proboscis elongation and passes through the circum-oesophageal nerve ring (together with the acinous salivary glands and radular sac) during development. The glandular folds (=glande framboisée) and rudimentary gland of Leiblein remain posterior to the nerve ring. The glandular folds are derived from the dorsal folds and the gland of Leiblein from the ventral strip which is rotated into a dorsal position by torsion. A finger-like evagination is formed which expands to form the gland and its duct.

Tracing the complete development of the venom gland in *Conus* was not possible, but early stages suggest a secretory region develops from the ventral strip and dorsal folds. In *Nucella* the same regions form the glandular oesophageal folds and the gland of Leiblein and these are believed to be the homologue of the venom gland and muscular bulb of the Conoidea. Both species exhibit striking developmental similarities. Features which are markedly different in their definitive state have identical ontogenetic origins and heterochrony of foregut development may explain the major differences observed in adult morphology.

RIASSUNTO I caratteri dell'apparato alimentare anteriore dei neogasteropodi sono stati ampiamente usati in analisi filogenetiche. Questi comprendono in particolare: le due paia di ghiandole salivari, la radula, la ghiandola e la valvola di Leiblein nei muricidi e l'apparato velenifero dei conoidei. Ipotesi e assunzioni sull'omologia di questi organi sia tra i Neogastropoda sia con altri gasteropodi possono essere verificate con studi ontogenetici.

Stadi di sviluppo in *Nucella lapillus* (Muricoidea) e *Conus anemone* (Conoidea) sono stati studiati con la ricostruzione di sezioni seriali (1µm - resin-embedded), microscopia ottica ed elettronica a scansione.

Sia in *Nucella* sia in *Conus* la massa boccale deriva da un'evaginazione ventrale dell'esofago. Una porzione anteriore forma la sacca sottolinguale in *Nucella* e il ceco radulare in *Conus*, mentre la porzione posteriore forma il sacco radulare. Le ghiandole salivari acinose, derivano come evaginazioni laterali della parete della cavità boccale. Questa regione si sposta dorsalmente in *Nucella* ed i dotti delle ghiandole salivari si accrescono posteriormente attaccati alla parete esofagea anteriore. In *Conus*, la cavità boccale è separata dall'esofago per mezzo del sacco boccale. Le ghiandole salivari acinose inizialmente si accrescono lateralmente dalla parete, quindi dorsalmente quando la regione secretoria si espande. I questo caso non divengono associate alla parete esofagea. Le ghiandole salivarie accessorie in entrambe le specie appaiono come evaginazioni appaiate del labbro ventrale, i dotti si accrescono posteriormente e terminano in aree secretorie. Durante lo sviluppo di *Nucella* i dotti si fondono, ma terminano in regioni secretorie appaiate. In *Conus*, le ghiandole appaiate si fondono completamente durante lo sviluppo, lasciando una singola ghiandola tubulare. La valvola di Leiblein in *Nucella* deriva dalle pieghe dorsali e dalla parete dorsale dell'esofago medio. Questa regione si differenzia prima dell'allungamento della proboscide e durante lo sviluppo passa attraverso l'anello nervoso circum-esofageo (assieme alle ghiandole acinose salivari e al sacco radulare). Le pieghe ghiandolari (=glande framboisée) e la rudimentale ghiandola di Leiblein rimangono posteriori all'anello nervoso. Le pieghe ghiandolari derivano dalle pieghe dorsali e la ghiandola di Leiblein dalla striscia ventrale che è ruotata per torsione in posizione dorsale. Si forma un'evaginazione digitiforme, che si espande a formare la ghiandola ed il suo dotto.

Tracciare il completo sviluppo della ghiandola del veleno in *Conus* non è stato possibile, ma gli stadi precoci suggeriscono che una regione secretoria si sviluppi dalla striscia ventrale e dalle pieghe dorsali. In *Nucella* le stesse regioni formano le pieghe ghiandolare esofagee e la ghiandola di Leiblein e queste sono ritenute essere omologhe alla ghiandola del veleno e al bulbo muscolare dei Conoidea. Entrambe le specie studiate mostrano rimarchevoli similarità nello sviluppo. Caratteristiche che sono marcatamente differenti nel loro stato definitivo hanno identiche origini ontogenetiche e fenomeni di eterocronia nello sviluppo dell'apparato alimentare anteriore possono spiegare le maggiori differenze nella morfologia adulta.

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INTRODUCTION

The Neogastropoda are generally regarded as monophyletic (Ponder 1973; Taylor & Morris 1988; Kantor 1996), but their relationships to other gastropods and relationships of the family groups within the order are highly uncertain (Ponder & Lindberg 1997).

The most important characters for phylogenetic studies of neogastropods are located in the alimentary system, particularly the foregut. Ponder (1973), defined four synapomorphies — two pairs of histologically distinct salivary glands located in front of the nerve ring, the mid-oesophageal valve of Leiblein, the mid-oesophageal gland and an anal gland - which he used to

divide the Neogastropoda into three superfamilies: the Muricoidea, Conoidea and Cancellarioidea. Recent studies have revealed a greater disparity in foregut anatomy (KANTOR 1991; TAYLOR, KANTOR & SYSOEV 1993) and KANTOR (1996) divided the Order into five suborders, three of these equivalent to PONDER's (1973) superfamilies, with the addition of the Olivelloidei and Pseudolivoidei.

The Neogastropoda have proven particularly intractable to phylogenetic analysis by either morphological or molecular methods. A recent cladistic analysis by PONDER and LINDBERG (1997) using only three neogastropod taxa, placed the Buccinidae as basal to the Muricoidea and Conoidea, which contradicts KANTOR (1996; this volume) who places Conoidea as basal to the Neogastropoda. HARASEWYCH, ADAMKEWICZ, BLAKE, SAUDECK, SPRIGGS, and BULT (1997) found that 18S rDNA sequences of taxa from about half the neogastropod families gave little resolution and whilst cytochrome C oxidase 1 sequence data produced resolved trees, these were characterised by low character indices and a high degree of homoplasy. Results so far however, reveal a lack of congruence with the morphological analyses.

One limitation for phylogenetic analysis of neogastropods is that little is known of the derivation and homologies of some foregut structures as related to other gastropods. Heterochrony was recently stressed as an important influence on the evolution of morphological trends in gastropods (PONDER and LINDBERG 1997) and ontogenetic studies are crucial for testing these ideas.

The present study is an attempt to bring together the results of work on two very different neogastropods; one, a muricoidean, relatively conservative in its foregut arrangement (*Nucella lapillus*), corresponding closely to the archetypal neogastropod form described by PONDER (1973), and the other a highly derived conoidean neogastropod with a foregut which exhibits extensive modifications from the archetypal state (*Conus anemone*). The aim is to determine whether common patterns exist in the development of key organ systems, and how the ontogeny of these organs produces the very different definitive morphologies found in these two species.

Nucella lapillus (Linnaeus, 1758), and *Conus anemone* Lamarck, 1810 are carnivorous species; *N. lapillus* is common on the Atlantic coasts of Europe and North America where it preys on sedentary bivalves and barnacles, whilst *C. anemone* lives on the intertidal reef platforms of the Indian Ocean shorelines of Western and South Western Australia (see RÖCKEL, KORN and KOHN, 1995 for details) and feeds on polychaetes.

The organogenesis of *N. lapillus* has been described by several authors (PORTMANN, 1925; PORTMANN and SANDMEIER, 1965), most notably STÖCKMANN-BOSBACH (1988, 1991) and STÖCKMANN-BOSBACH and FIORONI (1988) who investigated the pretorsional developmental stages. Post-torsional development, including organogenesis of the foregut, has been described by BALL (1994), BALL, TAYLOR and ANDREWS (1997) and BALL, ANDREWS and TAYLOR (1997).

There are only fragmentary accounts of the development of any members of the Conoidea. FIORONI (1965) described embryonic development of *Philbertia purpurea* (Montagu) (Conidae: Raphitominae) and there is an unpublished account of the development of *Conus mediterraneus* Bruguières by FRANC (1943) which includes some description of the development of the foregut and venom gland. Both accounts are, however, only sparsely illustrated. The larval development of *C. anemone* has also been studied using scanning electron microscopy to describe some of the external developmental features and to compare them with *N. lapillus* and *Conus dorreensis* Péron (BALL, 1999). The present paper expands on the latter work by investigating the internal anatomy through light microscopy of serial sections and computer-aided reconstructions.

METHODS

Collection of embryos

Nucella lapillus egg capsules were collected at Hayle Bay (Cornwall, U.K.). Identification was based on COSTELLO, DAVIDSON, EGGERS, FOX and HENLEY (1957) and on the presence of breeding females with the egg capsules.

Conus anemone egg capsules and adults were collected on Rottnest Island (near Perth, Western Australia) and identification was based on KOHN (1993) and SMITH, BLACK and SHEPHERD (1989).

After removal from the egg capsules using iridectomy scissors and pipettes, the embryos were examined to determine the approximate stage of development, narcotised and then fixed.

Specimen preparation

N. lapillus embryos were relaxed using 1% propylene phenoxytol in seawater. For *C. anemone* embryos, propylene phenoxytol was added in stages until relaxation was achieved. The amounts used varied with the stage of development. After relaxation, embryos were fixed using buffered glutaraldehyde and post-fixed for 1 hour in 1% osmium tetroxide.

Embryos were decalcified using saturated aqueous EDTA (disodium ethylene diamine tetra-acetic acid) to remove the larval shells and then dehydrated through ascending graded ethanol (acetone for *C. anemone*) prior to final preparation for electron microscopy or light microscopy.

Specimens for light microscopy (LM) were embedded in medium grade TAAB resin, re-orientated to give transverse sections and sectioned at 1µm intervals using an ultramicrotome. Slides were stained with toluidine blue in borax.

3D reconstruction

Transverse orientations were found to give the best results and serial sections were drawn using a *camera lucida*, and transferred to Jandell Scientific's PC3D computer program. This package was used to reconstruct the lateral viewpoints which were later redrawn by hand.

Scanning Electron Microscopy (SEM)

SEM specimens were critical point dried after dehydration and sputter coated with gold palladium. Philips XL30 FEG, Hitachi S800 and S2500 SEMs were used to examine the specimens.

Additional LM of adult *C. anemone* was carried out using 8µm wax-embedded serial sections stained with Haematoxylin and Eosin. Throughout the results, observations on adult anatomy are a combination of my own and Yuri Kantor's notes (Kantor, personal communication).

RESULTS

The anatomy of the adult *Conus anemone* is described and its development has been divided into 4 post-torsional stages (Stages I-III) summarised in two normal tables (Tables 1A and 1B).

Adult *Nucella lapillus* anatomy is not detailed here, since it has already been described by GRAHAM (1941) and MARTOJA (1971). Development has been described by STÖCKMANN-BOSBACH (1988; 1991); BALL (1994) and BALL *et al.* (1997a; 1997b). 11 developmental stages were recognised; 5 pre-torsional, 6 post-torsional. The post-torsional stages have been summarised here and compared to *C. anemone* in Table 2.

The developmental period described for *C. anemone* corresponds approximately to Stages 6-8 of *N. lapillus.* A different numbering system has been used for *C. anemone* to avoid confusion. Stages IA and IB can be regarded as early and late phases of Stage I, the differences are subtle and are based on observations of numerous specimens. There are however clear differences between Stages I and II and II and III.

Conus anemone

Adult proboscis

The proboscis is long and thin-walled with a basal rhynchodeal septum (Figure 1). The walls are formed from a layer of radial

Table 1A. Normal table for the development of *Conus anemone.*

External morphology

Stage / Feature	Stage IA	Stage IB	Stage II	Stage III
Protoconch	Small, 1 whorl, symmetrical aperture	Small, 1 whorl, symmetrical aperture	1_ whorls, asymmetrical aperture	1_ whorls, asymmetrical aperture
Velum	Bilobate, blunt, rounded lobes	Bilobate, blunt, rounded lobes	Bilobate, deeply notched	Collapsed against side of head
Propodium	Tiny/absent	Poorly-developed, finely ciliated	Well-developed, finely ciliated	Well-developed, finely ciliated
Metapodium	Well-developed, covered in ciliated papillae	Well-developed, covered in ciliated papillae	Elongate, waisted and pointed at posterior end, finely ciliated	Elongate, waisted and pointed at posterior end, finely ciliated
Operculum	Small, rounded	Small, rounded	Lens-shaped, elongate	Lens-shaped, elongate
Siphonal notch	Absent	Absent	Present	Present
Cephalocyst	Large, 2 cell types	Large, 2 cell types	Large, 2 cell types	Large, 2 cell types
Tentacles	Short, symmetrical in length, tipped with elongate cilia	Equal in length, longer than stage IA, tipped with elongate cilia	Elongate, narrow	Elongate, narrow
Tentacle bases	Small	Slightly enlarged	Bulbous, enlarged	Bulbous, enlarged
Anterior pedal mucus gland	Absent	Absent	Present	Present
Appearance of mouth	Narrow slit	Dorsal lips present, slightly enlarged	Dorsal lips enlarged to form buccal tube	Dorsal lips enlarged to form short proboscis
Proboscis sheath present?	No	No	Slight overgrowth dorsally	Dorsal overgrowth, ventral invagination
Behavioural development	Veliger	Veliger	Pediveliger	Hatchling

muscle fibres overlying a layer of longitudinal fibres. The proboscis retractor muscles occupy almost the whole volume of the proboscis. A snout gland is present on the right side of the rhynchodaeum in the form of a rounded sac filled with tall columnar cells which opens to the proboscis base via a wide duct. The rhynchodaeum is lined with a tall glandular epithelium.

Proboscis development

Proboscis development was not complete by the time of hatching and consequently could not be traced fully. Observations using SEM (Table 1A) showed that the initial elongation is due to the enlargement of the buccal lips to form a short snout (Figure 2A-C) (Stages I and II). This is followed by anterior growth of the body wall combined with dorsal and ventral invaginations to form the beginnings of a rhynchocoel (Figure 2D-E) (Stages II and III). The rhynchocoelic invaginations appear to be paired dorsally when they first appear and then fuse mid-dorsally to form a single curved dorsal invagination (Figure 3). Ventrally the invaginations are not so clearly defined but they are present in the later developmental stages (Stages II and III). The proboscis sheath continues to grow anteriorly as the tentacle bases fuse and begin to overgrow the proboscis base (Stage III). In sections (Table 1B), a fold near the base of the proboscis begins to develop and this is consistent with the rhynchodeal septum

Table 1B. Normal table for the development of *Conus anemone*.

Internal morphology

Stage/Feature	Stage IA	Stage IB	Stage II	Stage III
Position of buccal mass relative to cerebral commissure	Buccal mass posterior to cerebral commissure	Buccal mass posterior to cerebral commissure	Buccal mass level with cerebral commissure	Buccal mass anterior to cerebral commissure
Radular sac orientation relative to oesophagus	Radular sac short, lies ventral and parallel to oesophagus	Lies parallel to oesophagus, but alongside	Radular sac arches over cerebral commissure and tip lies anterior to cerebral ganglia	Radular sac penetrates nerve ring, lies approximately parallel with oesophagus
Radular teeth present?	Absent	Absent	Present	Present
Characteristics of radular sac	Flattened, posteriorly directed tube	Odontoblast nest appears	Odontoblast nest divided into two lateral regions by central ridge	Odontoblast nest divided into two lateral regions by central ridge, radular caecum present
Position of acinous salivary glands	Acinous salivary glands tubular, small and posteriorly directed	Start to point in opposite directions, still tubular, but lead to larger secretory areas	Point in opposite directions, left gland is anterior to nerve ring right gland penetrates it	Salivary glands prominent, tubular and anterior to nerve ring, ducts directed dorsally
Appearance of oesophagus posterior to buccal mass	Oesophagus short, before leading to stomach. Ventral and dorsal regions different	Oesophagus short, before leading to stomach. Ventral and dorsal regions different	Elongated, ventral region expanded, greenish granules present in cells	Elongated, thrown into longitudinal folds, venom gland separation begins. ventral part greatly expanded into W shaped lumen secretory granules shed into lumen
Appearance of accessory salivary glands	Accessory salivary glands short paired ducts	Difficult to determine if 2 ducts or one	Only one gland present, lumen is empty, no secretory cells	Lumen empty, no secretory granules, only one gland
Proboscis sheath present in sections?	No	Dorsal portion present	Dorsal and ventral components present	Dorsal and ventral components present
Proboscis present in sections?	No	No	Snout developing, with shallow sheath	Short proboscis present with dorsal sheath and ventral invagination
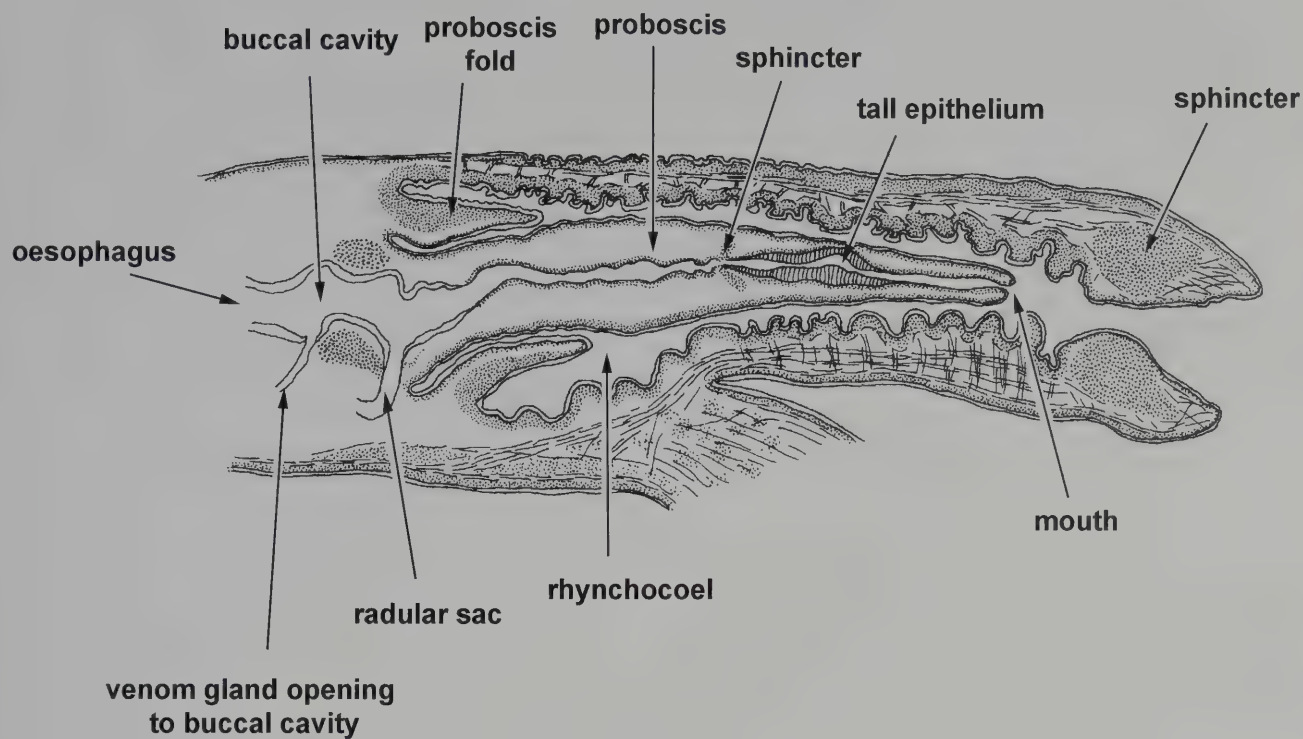
Figure 1. Longitudinal section through the proboscis and buccal mass of *Conus anemone*.

Table 2. Combined normal table comparing the development of *Nucella lapillus* with that of *Conus anemone*.

Nucella lapillus developmental stage	*Conus anemone* developmental stage
Stage 6 Post-torsional veliger	Stages IA and IB
Visceral mass rounded	Visceral mass rounded
Foot much smaller than visceral mass	Foot smaller than visceral mass
Shell covers visceral mass	Shell covers visceral mass
Tentacle rudiments appear	Tentacles present
Velar lobes simple and rounded	Velar lobes present, bilobed
Velum large relative to visceral mass	Velum large relative to visceral mass
Post-torsional	Post-torsional
Stage 7 Early pediveliger (veliconcha)	**Stage II**
Visceral mass begins to coil	
Foot larger than visceral mass	Foot larger than visceral mass
Foot functional, pediveliger	Foot functional, pediveliger
Enlarged propodium	Enlarged propodium
Velum much smaller than visceral mass	Velum still relatively large
Pallial organs begin to develop	
Siphonal notch present	Siphonal notch present
Early stages of proboscis development	First stages of proboscis development
Stage 8 First crawling stage	**Stage III**
Shell calcified	
Velar lobes resorbed	Velar lobes partially or wholly resorbed
Proboscis develops	Early proboscis development

found in the adult (compare Figs. 1 and 3). Thus the proboscis forms by elongation of the oral tube and body wall surrounding the mouth, whilst the proboscis sheath appears to grow by elongation of the body wall both anterior to and posterior to the tentacles.

Adult buccal mass

The buccal mass is muscular and lies at the base of the proboscis anterior to the nerve ring (Figure 1). The buccal tube passes from the mouth to the buccal mass and then continues posteriorly into the oesophagus whilst the venom gland enters the buccal mass ventrally on the right side. The buccal cavity lies ventral to the oesophagus and is composed of the buccal sac (where the ducts of the acinous salivary glands enter the buccal cavity laterally) and the radular sac and radular caecum (Figure 4). In *C. anemone*, the radular sac is rotated through 90° (the rotation occurring within the buccal sac) and the radular caecum lies almost directly below the buccal sac, whilst the radular sac lies to the left of the oesophagus and arches dorsally so that the odontoblast nest lies on the left extremity of the haemocoel dorsal to the oesophagus.

Buccal mass development

In the earliest embryos examined (Stage IA), the buccal mass lies just behind the level of the statocysts, posterior to the circum-oesophageal nerve ring (composed of the cerebral, pleural and pedal ganglia)(Figure 5A). The buccal commissure, which passes between the radular sac and the ventral part of the buccal mass and oesophagus, is also posterior to the nerve ring. This *embryological* condition is the reverse of adult neogastropods where the buccal mass and buccal ganglia lie anterior to the nerve ring (Ponder, 1973).
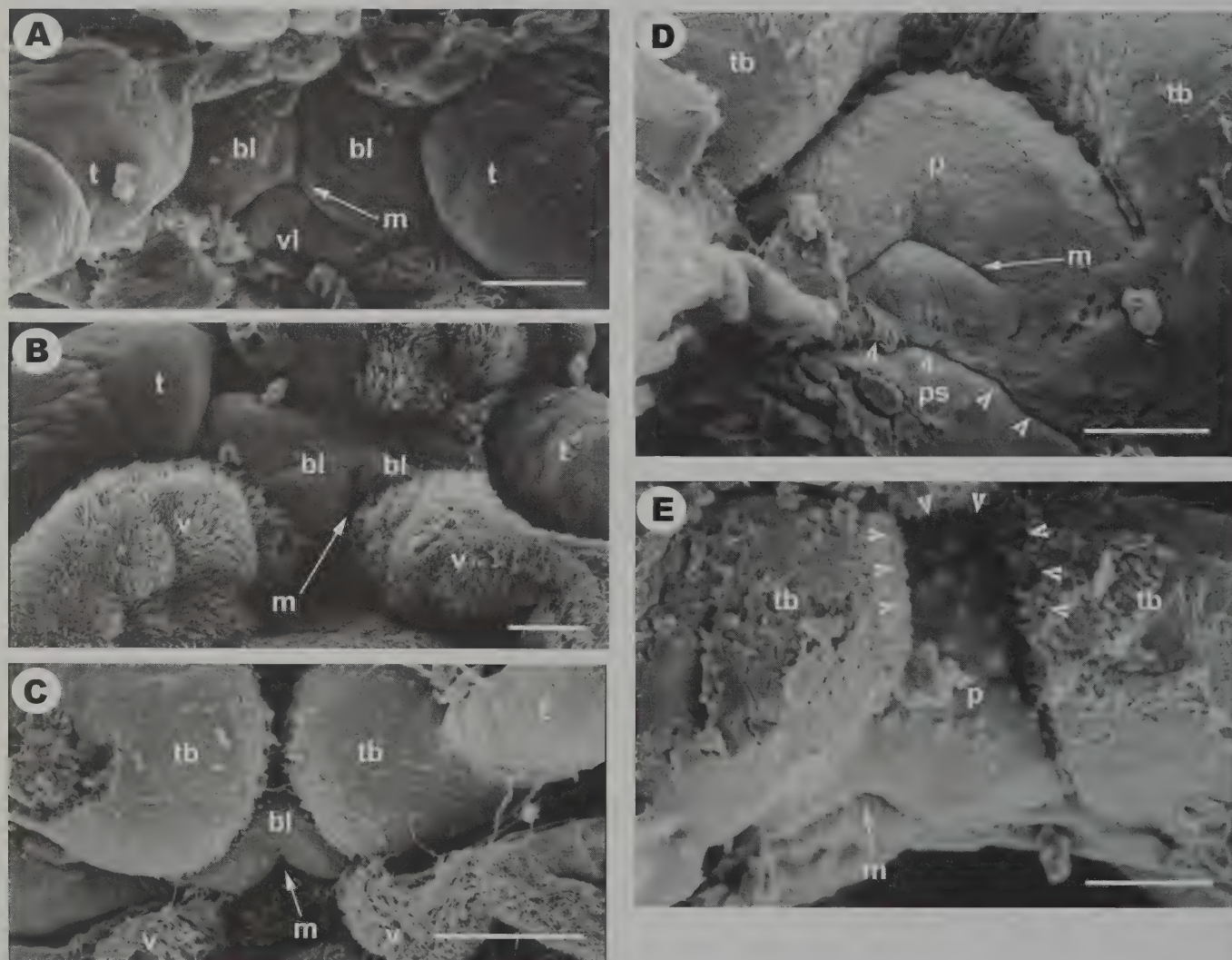
Figure 2. Development of the proboscis in *Conus anemone* observed using SEM. **A.** Stage IA. Buccal and ventral lips discernible, but not enlarged. Scale bar 25µm. **B.** Stage IB. Enlargement of buccal lips. Scale bar 30µm. **C.** Stage II. Buccal lips form distinct fold. Tentacle bases overgrow developing snout. Scale bar 50µm. **D.** Stage III. Oral tube and buccal lips form proboscis rudiment. Tentacle bases cover lateral and dorsal surfaces of proboscis. Lateral proboscis sheath absent due to presence of velar lobes. Ventral invagination present below mouth (arrow heads). Scale bar 20µm. **E.** Stage III, dorsal view. Tentacle bases overgrow proboscis. Growth direction indicated by arrowheads. Scale bar 20µm. (Key to lettering: bl-buccal lips; m-mouth; p-proboscis rudiment; ps-proboscis sheath; t-tentacle; tb-tentacle bases; v-velum; vl-ventral lip).
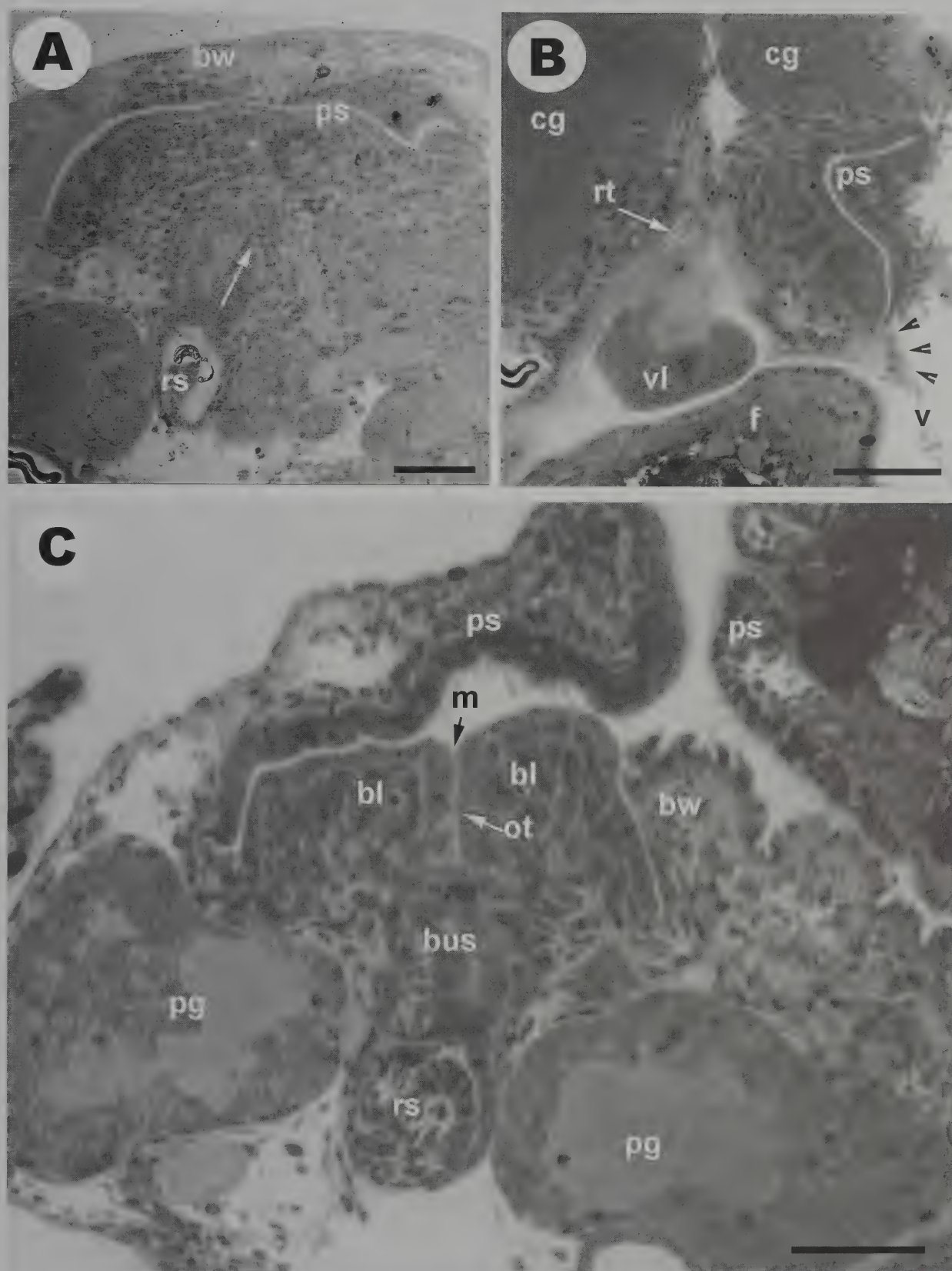
Figure 3. Development of the proboscis and proboscis sheath in *Conus anemone* observed using light microscopy. **A.** Stage II, oblique-transverse section through head. Arrow indicates path of oesophagus towards mouth. Scale bar 50μm. **B.** Stage III, transverse section level with mouth. Arrowheads mark point where invaginations interrupted by velum. Note radular tooth held at ventral lip. Scale bar 50μm. **C.** Stage III, horizontal longitudinal section. Proboscis viewed from above. Proboscis sheath covers developing proboscis. Scale bar 50μm. (Key to lettering: bl-buccal lip; bus-buccal sac; bw-body wall; cg-cerebral ganglion; f-foot; m-mouth; ot-oral tube; pg-pedal ganglion; ps-proboscis sheath; rs-radular sac; rt-radular tooth; v-velum; vl-ventral lip)

Four distinct stages of development of the buccal mass are described (Table 1B). Embryos at stage IA have a buccal mass consisting of a shallow buccal cavity, with short, paired salivary ducts, and a radular diverticulum (Figs. 5A and 6A). The radular sac, which has already formed in a previous, but unobserved, developmental stage, is short and poorly differentiated and lies parallel to the oesophagus pointing posteriorly (Figure 6A). It's lumen is dorso-ventrally flattened and lacks both radular teeth and an odontoblast nest.

In transverse section, the oesophagus at the level of the buccal mass is clearly divisible into a dorsal strip, composed of ciliated and mucus-secreting cells, and the ventral buccal cavity whose cells stain more densely and appear to be unciliated (Figure 5A). These cells form the lateral and ventral walls of the buccal cavity and also pass posteriorly into the next part of the oesophagus. The ventral part of the buccal cavity is dorso-ventrally flattened and towards the posterior of the cavity, paired lateral evaginations, which are the ducts of the acinous salivary glands, arise from the lateral walls (Figure 5A).

Ventral to the buccal cavity lies a thick mass of apparently undifferentiated tissue, this corresponds to the buccal musculature in other gastropod embryos (Figure 5A). There is a clearly defined muscle which originates within this mass and passes anteriorly through the nerve ring above the pedal commissure and merges with the floor of the haemocoel. Similarly, a posteriorly-directed muscle leaves this mass and merges with the pedal musculature posterior to the nerve ring (Figure 5D).

As the radular sac elongates (Stage IB), it remains approximately parallel to the path of the oesophagus, although it is deflected to the left and its posterior limit lies slightly dorsal to the oesophagus (Figs. 5B and 6B). The buccal mass still lies ventral to the oesophagus but has become slightly deflected to the left and twisted clockwise (when viewed from above) (Figure 5B). The twisting motion of the radular sac affects the lower part of the buccal cavity where the ducts of the salivary glands arise. As a result, the ducts no longer emerge at right angles to the oesophagus. This is seen to a greater degree in the next stage of development (Stage II)(Figure 6C).

The specialised characters of the conoidean buccal mass begin to appear in stage II. The buccal sac extends ventrally, further separating the radular sac from the oesophagus. As a consequence, the part of the buccal wall which includes the salivary gland ducts becomes recognisable as the buccal sac and the region postero-ventral to it becomes the definitive radular sac (sometimes referred to as the "long arm" of the radular sac) (Figure 5B). The radular sac rotates through approximately 90° with respect to the oesophagus and undergoes considerable growth. The salivary gland ducts show the effects of rotation as they now exit the buccal wall parallel to the oesophagus, but in opposite directions to each other (Figure 6C). This process is much more pronounced than in stage I. The right salivary gland is posteriorly directed and protrudes into the cephalic haemocoel behind the nerve ring, whilst the left salivary gland passes around the left side of the oesophagus and then anteriorly so that its tip lies within the nerve ring just anterior to the cerebro-pleural commissures.

The anterior portion of the radular sac has not yet become differentiated to form the radular caecum (= "short arm", the homologue of the sub-lingual pouch). However, the radular sac has grown in length so that it passes from behind the nerve ring arches dorsally above the oesophagus and circum-oesophageal nerve ring. Its tip passes between the cerebral ganglia and over the cerebral commissure and lies anterior to the nerve ring (Figure 5C-D).

Figure 4. A. Diagrammatic view of generalised *Conus* foregut (modified after Wilson and Gillet 1979, Figure 4). B. Sketch diagram of dissection of adult *Conus anemone*. Scale bar 5mm. (Key to lettering: sd-salivary gland ducts (cut)).

During stage II, the internal structure of the radular sac also undergoes structural changes; the odontoblast nest (which first appears at Stage IB) has differentiated to form two distinct lateral regions separated by a low ridge. This arrangement seems to have formed through lateral expansion of the walls of the radular sac (Figure 5D). The radular sac now contains numerous radular teeth which are produced in two rows from the separate left and right portions of the odontoblast nest (Figure 5D). Each enrolled tooth is formed around a single cell. The teeth have recognisable barbs and are present within the whole length of the radular sac. Attempts to isolate larval teeth for SEM examination were unsuccessful.

At the crawlaway stage (Stage III - the oldest embryos examined), the anterior portion of the radular sac has expanded and differentiated to form the radular caecum (Figure 6C). Radular teeth stored within it are present in a distinctly different orientation from those remaining in the radular sac and appear to have undergone a 180° rotation from the radular sac to the cae-

cum. The most significant point is that the buccal mass (including the radular sac and the acinous salivary glands) now lies anterior to the nerve ring. This displacement coincides with the elongation of the snout as the proboscis begins to form

Adult acinous salivary glands

The paired acinous salivary glands form a single mass of interdigitating secretory tissue in the dorsal portion of the cephalic haemocoel anterior to the nerve ring. The glands are acinous in nature each consisting of a branching alveolar-like network leading to a single duct. A pair of ciliated ducts pass from the ventral surface of each gland to the buccal sac where they merge with the wall of the buccal cavity. Since the ventral part of the buccal mass in *C. anemone* is orientated at 90° to the long axis of the oesophagus the ducts enter the buccal cavity anteriorly and posteriorly with respect to the long axis of the animal.
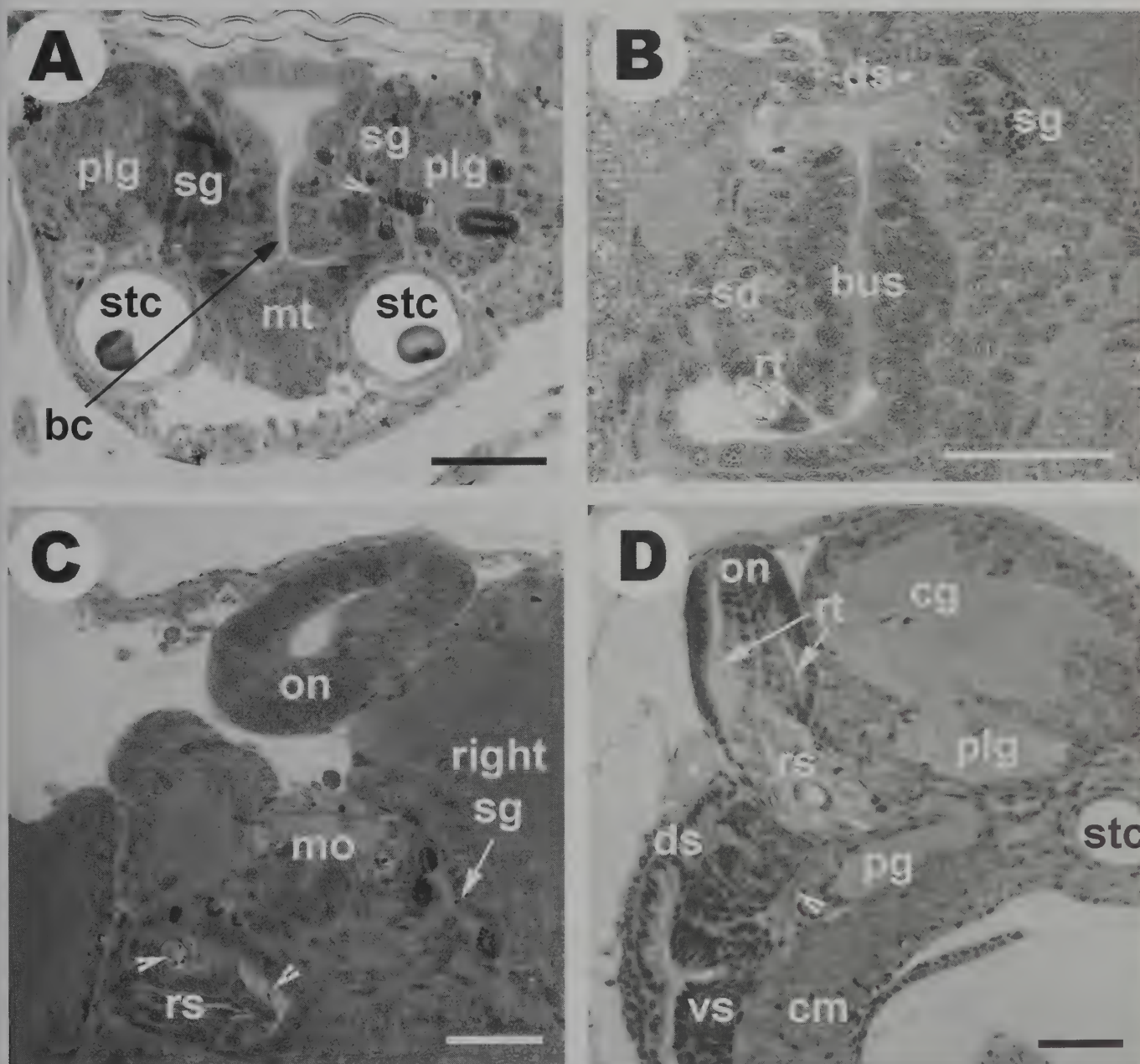
Figure 5. Development of the buccal mass in *Conus anemone* observed using light microscopy. **A.** Stage I, transverse section. Buccal cavity and acinous salivary glands, lying parallel to oesophagus are visible. Buccal mass posterior to nerve ring. Scale bar 50µm. **B.** Stage II, transverse section. Buccal sac and deflection of radular sac. Only right acinous salivary gland visible with left duct. Scale bar 50µm. **C.** Stage II, transverse section. Section posterior to buccal mass showing odontoblast nest and posteriorly directed right acinous salivary gland. Scale bar 50µm. **D.** Stage II, longitudinal section. Radular sac and odontoblast nest. Mid-oesophagus shows ciliated dorsal strip and secretory ventral strip. Buccal mass lies anterior to nerve ring, but is not visible in this plane of section. Arrow indicates retractor muscle passing through nerve ring. Star indicates supra-oesophageal connective. Scale bar 50µm. (Key to lettering: bus-buccal sac; cg-cerebral ganglion; cm-columellar muscle; ds-dorsal strip; mo-mid-oesophagus; mt-mesodermal tissue; on-odontoblast nest; pg-pedal ganglion; plg-pleural ganglion; rs-radular sac; rt-radular tooth; sd-acinous salivary gland duct; sg-acinous salivary gland; stc-statocyst; vs-ventral strip)

Acinous salivary gland development

The acinous salivary glands arise from dorso-lateral evaginations of the wall of the buccal mass (Table 1B; Stage IA) and their initial growth is directed posteriorly parallel to the oesophagus mid-line (Figure 5A).

The ventral growth of the buccal mass and its subsequent rotation during development (stages IB-II) means that the ducts no longer both grow posteriorly, but instead the left duct grows anteriorly and the right duct grows posteriorly (Figure 5B-C). Since the ducts are not associated with the walls of the oesophagus the glands are free to grow into the space available to them between the circum-oesophageal ganglia (nerve ring) and the oesophagus (Figure 5B-C). The growth of the left gland is constricted by the posterior wall of the rhynchodaeum and becomes folded over; the right gland is obstructed by the ganglia of the nerve ring and is also folded. Dark-blue staining granules begin to appear in the cytoplasm of the cells forming the terminal secretory regions which surround the ciliated, tubular ducts (Figure 5B).

This pattern of growth continues in the next developmental stage (Stage II); the anteriorly directed left salivary gland is compressed against the body wall, whilst the right salivary gland penetrates the nerve ring and expands posterior to it. In stage III, the glands lay anterior to the nerve ring, were clearly tubular and not yet strongly arborescent.

Adult accessory salivary glands

Only a single accessory salivary gland and its duct were observed. The duct could not be traced through the thick wax sections, but is presumed to open to the ventral lip as is the case in other neogastropods. Its appearance was similar to that described in *Conus flavidus* and *Conus vexillum* by Schultz (1983) and to adult *N. lapillus* (*see* Andrews 1981), a thin muscle layer lay between two layers of secretory cells which form a pseudo-stratified epithelium.

Accessory salivary gland development

The first developmental stage shows that the accessory salivary glands arise as paired evaginations of the ventral lip of the mouth (Stage IA)(Figure 7C). This region is recognisable since it has smaller, more densely staining cells than those of the oral tube or oesophagus (Figure 7D). The unciliated ducts appear to be filled with microvilli and are 60-100µm long. They can be traced for a short distance posteriorly along the ventral surface of the oral tube, before they terminate.

In subsequent stages only a single duct could be traced. The opening to the ventral lip appeared wider in stage IB than in the previous stage and it is likely the two ducts have fused. The single duct has a length of approximately 100µm and the termination is distinctly rounded at 30µm in diameter compared to a duct diameter of 10µm.

In stage III the gland is larger and has a defined sub-epithelial layer with a gap and then a layer of epithelial cells (Figure 7E). The necks of some epithelial cells can be seen to pass the gap and open to the lumen. Muscle cells which fill the gap in the adult are not distinguishable in the embryonic stages. The gland cells have a finely granular cytoplasm, but no secretory granules.
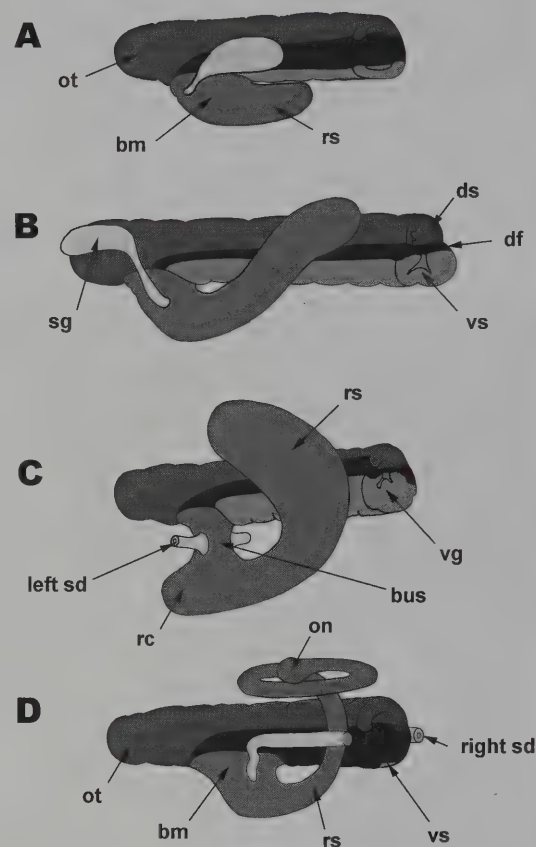
Figure 6. Diagrams comparing the development of the buccal mass and radular sac in *Conus anemone* with *Nucella lapillus.* **A.** *C. anemone* stage IA. Buccal mass and radular sac present. Acinous salivary glands and radular sac lie parallel to oesophagus. Ventral strip poorly developed. **B.** *C. anemone* stage IB. Radular sac has grown dorsally and twisted to the left. Left acinous salivary gland grows anteriorly, right (not shown) grows posteriorly. Dorsal strip has numerous longitudinal folds, ventral strip highly glandular. **C.** *C. anemone* stage III. Radular sac arches dorsally, anterior portion lies anterior to nerve ring (not shown). Buccal sac and radular caecum formed. Separation of venom gland from ventral strip has begun. **D.** *N. lapillus,* stage 10. Radular sac grows around left side of oesophagus and curves dorsally. Acinous salivary gland ducts pass posteriorly parallel to oesophagus. Ventral strip undifferentiated. (Key to lettering: bm-buccal mass; bus-buccal sac; df-dorsal fold; ds-dorsal strip; on-odontoblast nest; ot-oral tube; rc-radular caecum; rs-radular sac; sd-acinous salivary gland duct; sg-acinous salivary gland; vg-venom gland rudiment; vs-ventral strip)

Adult venom apparatus and mid-oesophagus

The mid-oesophagus commences just posterior to the buccal mass where a muscular sphincter is located. Just posterior to this sphincter the venom gland opens to the posterior right side of the oesophagus (Figure 1). The venom apparatus is well-developed with a highly coiled venom gland and a large oval, muscular bulb lying at the extreme posterior of the haemocoel with the anterior portion (leading to the venom gland) pointing ventrally (Figure 4). The bulb is large, occupying approximately half of the haemocoel and has two longitudinal muscle layers divided by a thin layer of connective tissue. In dissected anaesthetised

specimens, the bulb could be observed to pulse as it contracted. Each contraction drew the distal portion of the venom gland into the bulb as the bulb contracted longitudinally.

Venom apparatus development

In stage I the oesophagus posterior to the buccal mass is divisible into a ciliated dorsal strip and a ventral region composed of dense unciliated cells (Figure 8). This part of the oesophagus is short and at this stage leads to the stomach with little other differentiation.

In stage II, the anterior part of the oesophagus, near to the buccal mass, is tubular and divisible dorso-ventrally into a dorsal strip comprising mucous-secreting goblet and ciliated cells, and a ventral region of undifferentiated, unciliated cells (Figure
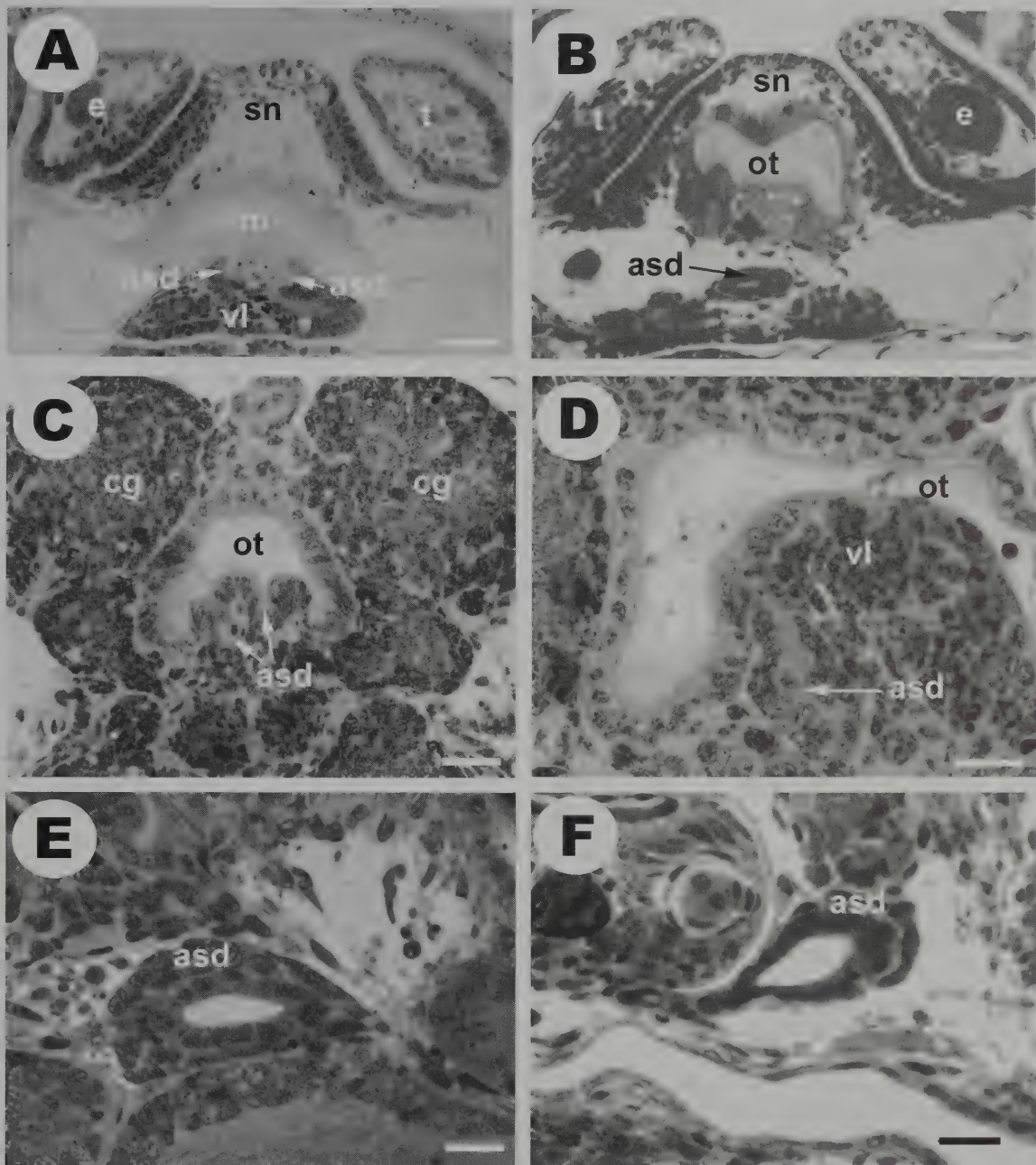
Figure 7. Development of the accessory salivary glands in *Conus anemone* and *Nucella lapillus* observed using light microscopy. **A.** *N. lapillus,* stage 7. Transverse section level with mouth. Note paired invaginations of accessory salivary gland ducts (arrowed). Scale bar 20µm. **B.** *N. lapillus*, stage 7. Transverse section posterior to mouth. Accessory salivary gland ducts fused, but lumen still paired. Scale bar 20µm. **C.** *C. anemone*, stage IA. Transverse section through oral tube. Note paired accessory salivary gland ducts. Scale bar 20µm. **D.** *C. anemone*, stage IB. Longitudinal section through oral tube. Accessory salivary gland duct lies further from mouth than *N. lapillus*. Scale bar 20µm. **E.** *C. anemone*, stage III. Accessory salivary gland showing two cell layers, muscle cells form central layer. Scale bar 10µm. **F.** *N. lapillus*, stage 7. Accessory salivary gland showing two secretory cell layers. Muscle cells appear between them. Scale bar 15µm. (Key to lettering: asd-accessory salivary gland duct; cg-cerebral ganglion; e-eye; m-mouth; ot-oral tube; sn-snout; t-tentacle; vl-ventral lip).

8). Posterior to this region, level with the oesophageal ganglia, the pleuro-visceral connectives cross over the oesophagus and the anterior aorta passes below it. These features would normally define it as the mid-oesophagus in adult neogastropods. At this point the oesophagus has three distinct dorso-ventral divisions; the dorsal strip is present dorsally, ventral to it on either side lie densely staining, ciliated cells which are probably homologous to the dorsal folds of the Muricoidea (Figure 8). The ventral region is glandular, unciliated and consists of tall cells containing fine green granules. This ventral region is
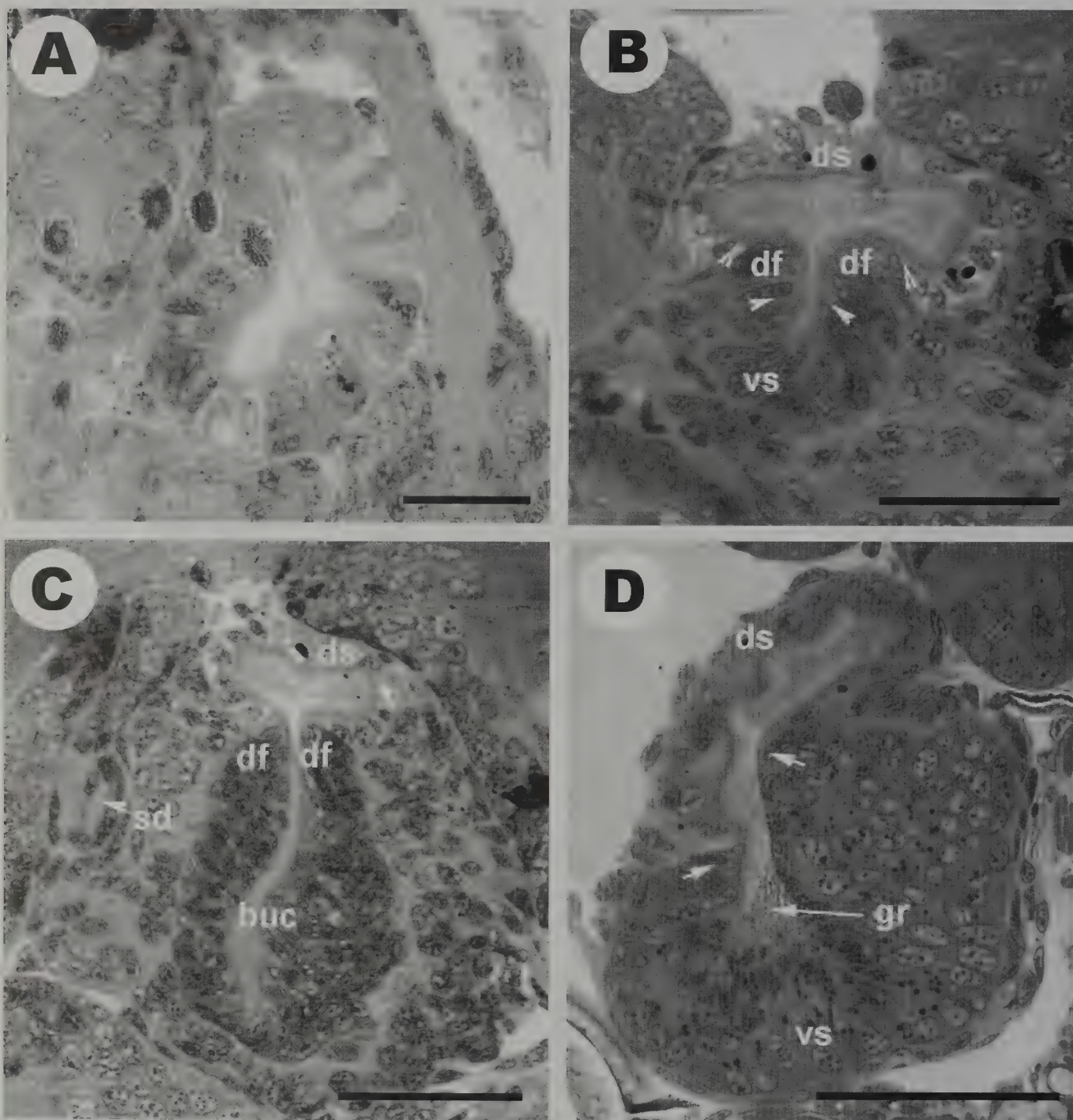
Figure 8. Development of *Conus anemone* mid-oesophagus observed using light microscopy. **A.** Stage IA, transverse section. Showing undifferentiated mid-oesophagus composed of ciliated and mucous-secreting cells. Scale bar 25µm. **B.** Stage IB, transverse section. Mid-oesophagus has differentiated close to buccal mass to form dorsal strip, ciliated dorsal folds (arrows indicate limits) and unciliated ventral strip. Scale bar 25µm. **C.** Stage II, transverse section. Ciliated dorsal folds close to unciliated buccal sac. Scale bar 50µm. **D.** Stage III, transverse section. Highly glandular ventral strip posterior to buccal mass. Dorsal folds reduced (arrowed), and ventral to dorsal strip. Note granules shed into oesophageal lumen. Scale bar 50µm. (Key to lettering: buc-buccal sac; df-dorsal folds; ds-dorsal strip; gr-secretory granules; vs-ventral strip)
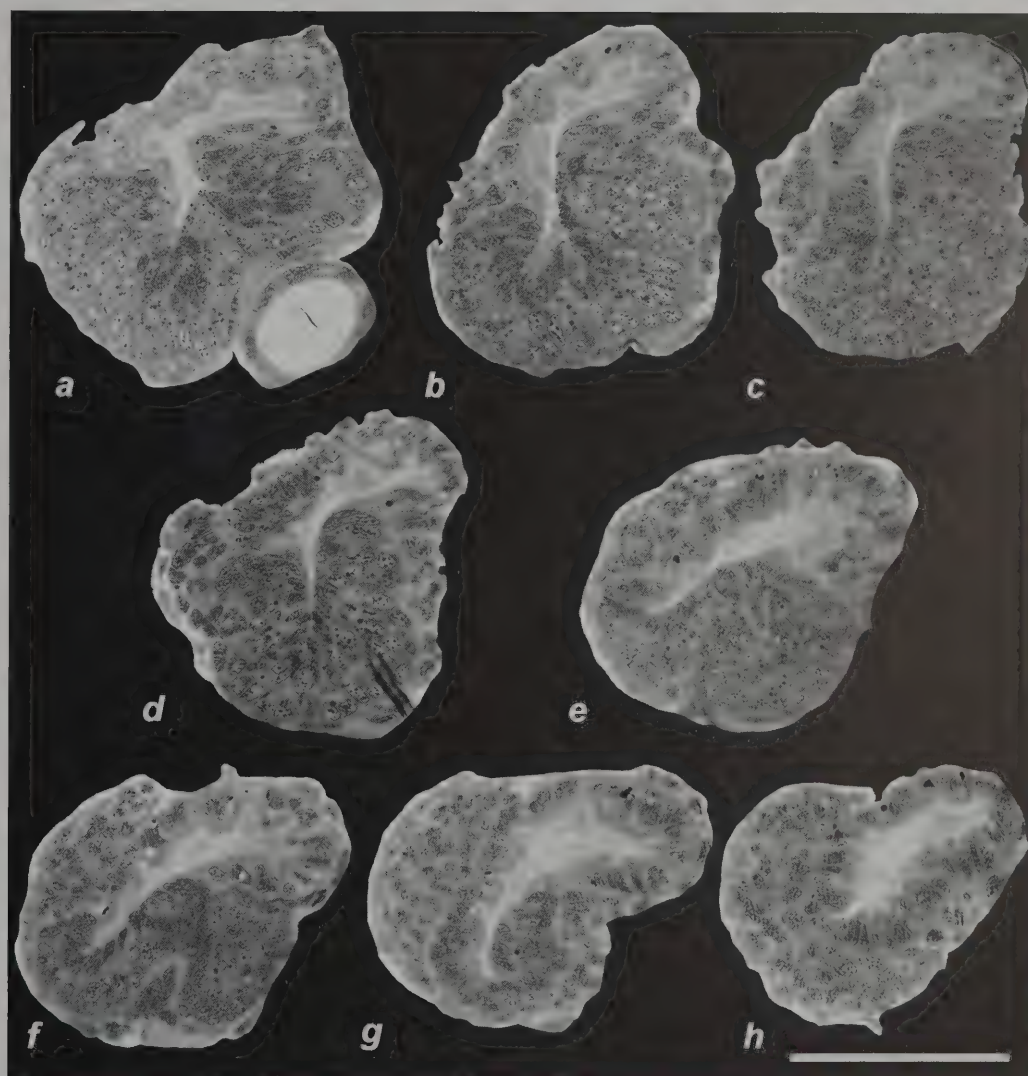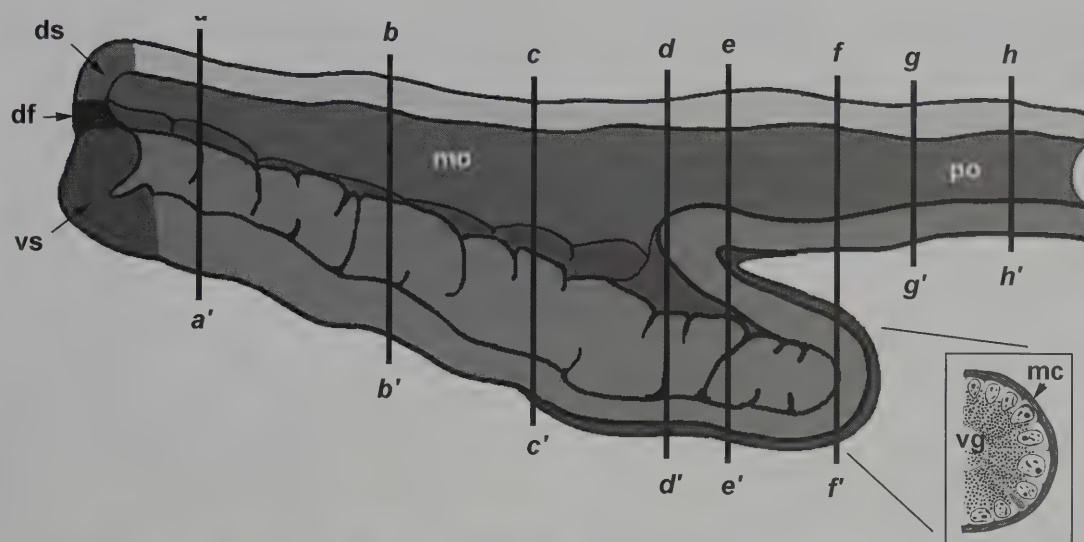
Figure 9. Reconstruction of the development of the venom gland in *Conus,* observed using light microscopy. Sections a-h transverse sections through mid-oesophagus at positions shown (a-a' to h-h') in diagrammatic reconstruction. Sections a-d show height increase in lumen. Sections e and f show dorsal portion of the oesophagus separated to form an outpushing containing secretory cells. Sections g and h show secretory cells absent from posterior oesophagus. Boxed diagram shows detailed reconstruction of tip of outpushing. Sections e and f show mixture of granules and nuclei in glancing section through base of columnar cells. Scale bar 50µm. (Key to lettering: df-dorsal folds; ds-dorsal strip; mc-muscle cells; mo-mid-oesophagus; po-posterior oesophagus; vg-venom gland; vs-ventral strip)

thrown into longitudinal folds and in transverse section has a shallow, w-shaped cross section. The secretory cells line the ventral side of the oesophagus and terminate at a point near to where the oesophagus enters the stomach (Figure 8). At this stage in development, the oesophagus has undergone a little over 90° of torsion and has become longitudinally folded and thrown into deep lateral folds. The glandular region lies entirely posterior to the nerve ring, and commences immediately posterior to the buccal mass (Figure 5D).

By stage III, the unciliated ventral glandular region has expanded and the ciliated cells (dorsal fold homologue) between the dorsal strip and the ventral glandular region have largely vanished. The secretory cells have begun to shed large, greenish granules into the lumen (Figure 8). This is particularly apparent towards the posterior limit of this part of the oesophagus. Granules fill the lumen of the dorsal part of the oesophagus.

Within the glandular region, the ventral secretory region bulges conspicuously and a finger-like projection is formed from the ventral wall of the oesophagus. Posteriorly, the oesophagus narrows again, but the ventral secretory cells are largely absent from the oesophagus walls (Figure 9). Detailed inspection of transverse sections reveals that at the end of the
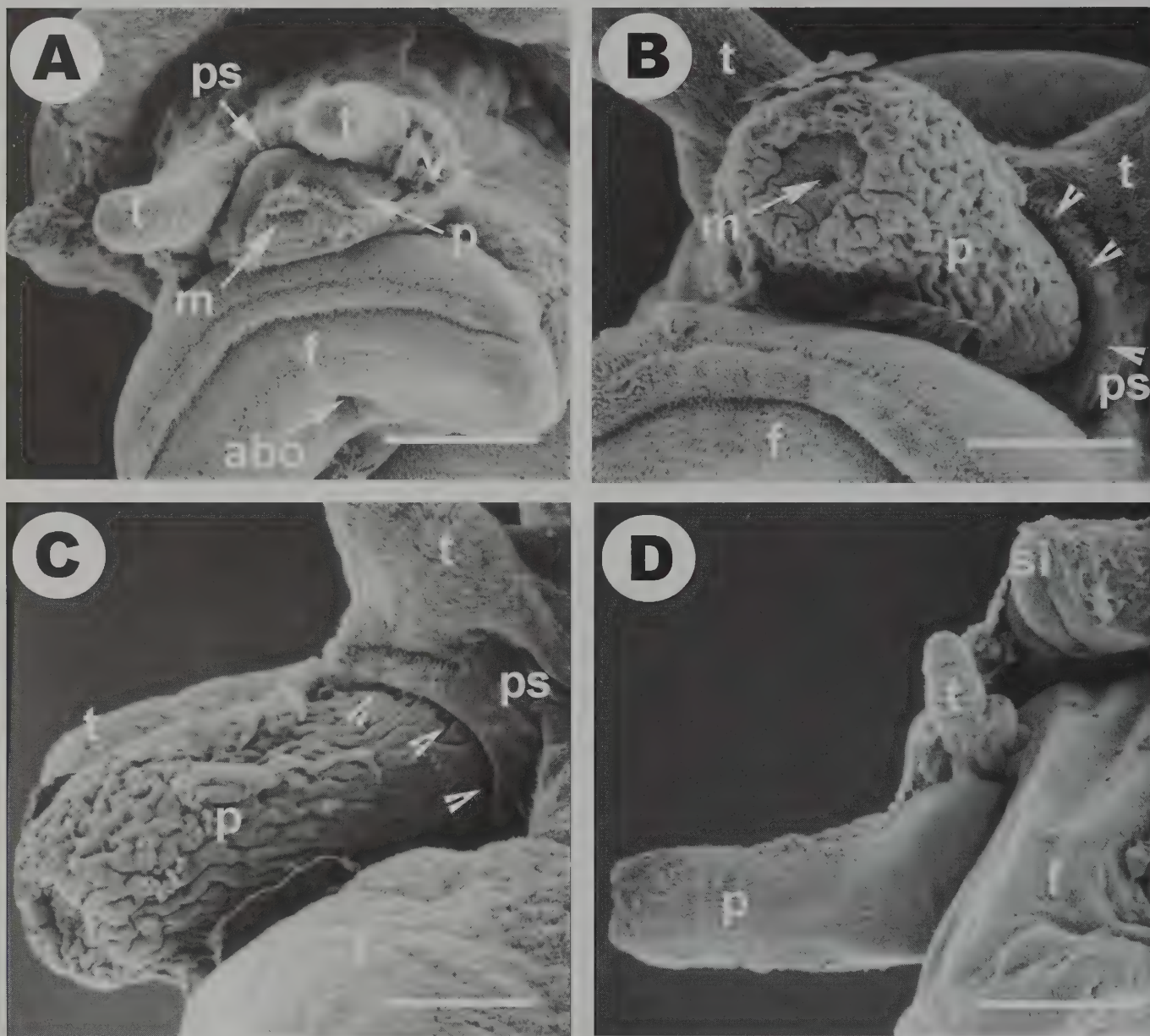
Figure 10. Proboscis development in *Nucella lapillus* observed using SEM. **A.** Stage 7, veliger. Proboscis rudiment overgrown by tentacle bases and proboscis sheath. No lateral proboscis sheath due to presence of velar lobes. Scale bar 100µm. **B.** Stage 8, post-veliger. Proboscis capable of limited extension. Base surrounded by proboscis sheath (indicated by arrowheads). Scale bar 75µm. **C.** Stage 8 post veliger, lateral view. Proboscis sheath indicated by arrowheads. Note smooth epithelium at th base of proboscis. Scale bar 75µm. **D.** Crawlaway stage. Note long proboscis, with smooth base and relatively smaller tentacles. Oesophageal loop well formed at this stage. Scale bar 200µm. (Key to lettering: abo-accessory boring organ; f-foot; m-mouth; p-proboscis; ps-proboscis sheath; si-siphon; t-tentacle)
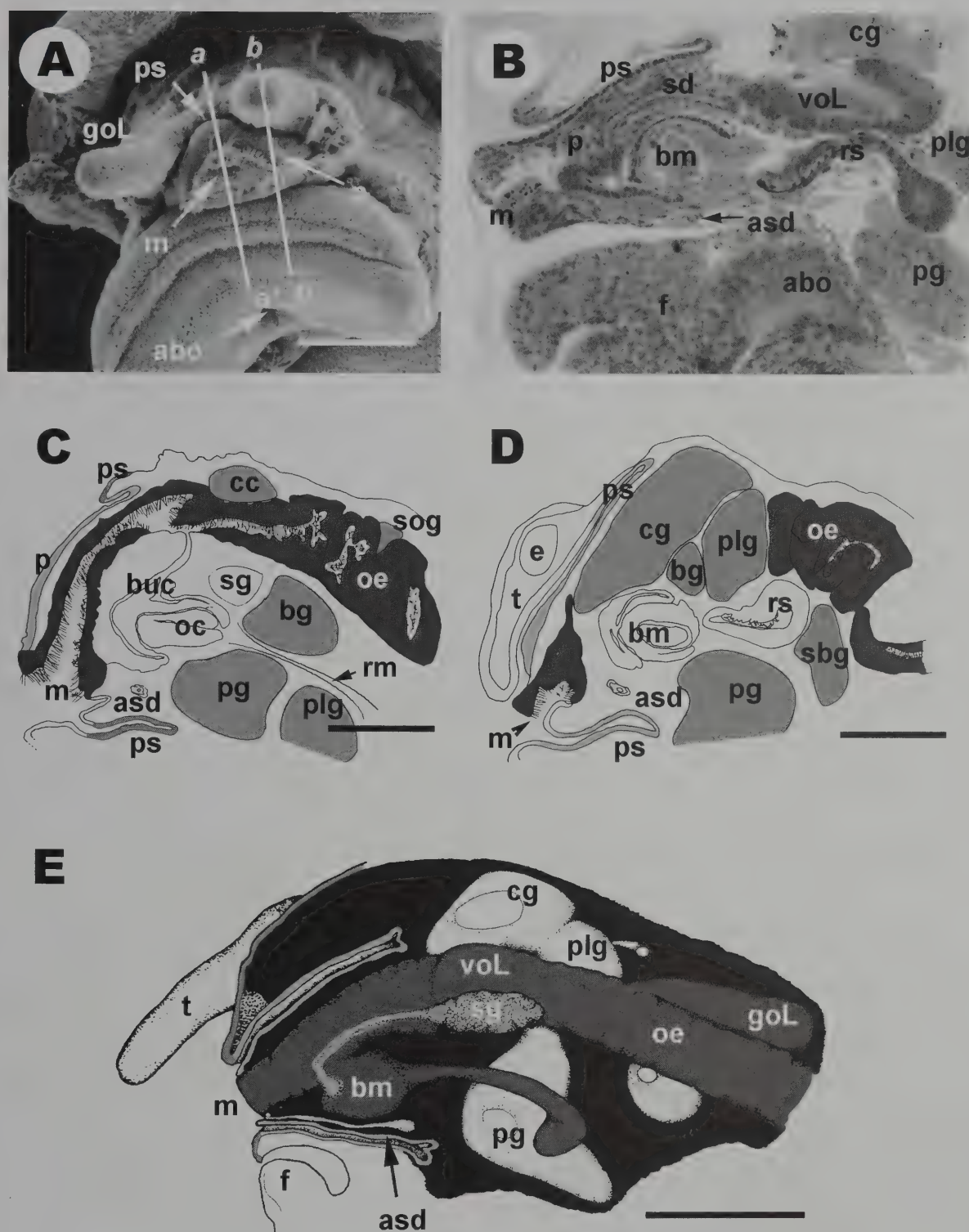
Figure 11. Proboscis development in *Nucella lapillus*, observed using SEM and light microscopy. **A.** Stage 7, frontal view. Showing well-developed proboscis rudiment overgrown by tentacle bases and proboscis sheath. Lines a-a' and b-b' refer to longitudinal sections C and D respectively. Scale bar 100μm. **B.** Stage 8, post veliger, longitudinal section, retracted proboscis. Note proboscis sheath formed by thinner infolded body wall. No oesophageal loop present at this stage. Scale bar 75μm. **C.** Stage 7, diagrammatic section through line a-a'. Exposed dorsal wall of proboscis rudiment covered at base by developing proboscis sheath. Oesophagus folded, but does not form loop. Note odontophoral retractor muscle passing through nerve ring. Scale bar 50μm. **D.** Stage 7, diagrammatic section through line b-b'. Showing lateral portions of proboscis rudiment covered by tentacle bases forming lateral proboscis sheath. Note radular sac penetrates nerve ring. Scale bar 50μm. **E.** Stage 8, longitudinal reconstruction. Note valve of Leiblein lies anterior to nerve ring. Gland of Leiblein has begun to separate from mid-oesophagus. Scale bar 75μm. (Key to lettering: abo-accessory boring organ; asd-accessory salivary gland duct; bg-buccal ganglion; bm-buccal mass; buc-buccal cavity; cc-cerebral commissure; cg-cerebral ganglion; e-eye; f-foot; goL-gland of Leiblein; m-mouth; oc-odontophoral cartilage; oe-oesophagus; p-proboscis; pg-pedal ganglion; plg-pleural ganglion; ps-proboscis sheath; rm-odontophoral retractor muscle; rs-radular sac; sbg-sub-oesophageal ganglion; sd-acinous salivary gland duct; sg-acinous salivary gland; t-tentacle; voL-valve of Leiblein)

evagination, the cells are predominantly in TS and the nuclei are wholly surrounded by granules. This suggests that the evagination terminates as a blunt tube (Figure 9).

Nucella lapillus

Proboscis development

The pleurembolic proboscis begins its development as an enlargement of the pre-tentacular region of the head, forming a short snout (Figure 10A). At this stage the reduced velar lobes are still present (Stage 7). Dorsal and ventral invaginations anterior to the tentacles form the first stages of the rhynchocoelic cavity. These invaginations are initially paired dorso-laterally and ventro-laterally but link at their dorsal mid-line to form an invaginated arc which divides posteriorly (Figure 11C-D). The dorsal and ventral components are separated at the level of the velar lobes by the velar cilia which pass to the mouth (Figure 10A).

Progressive increase in the length of the snout, to form the proboscis, accompanied by resorption of the velum and increase in the depth of the rhynchocoel forms the proboscis and its sheath (Stage 8)(Figure 10B-C). As the snout grows, an important factor in proboscis sheath formation is the anterior growth of the pre-tentacular epithelium which grows anteriorly to cover the developing proboscis, and the fusion of the tentacle bases along their mid-dorsal line (Figure 11). By stage 8, and well in advance of the hatching stage 11, the proboscis is capable of protrusion and at this stage in development profound internal changes are noted as the whole of the anterior oesophagus and the first part of the mid-oesophagus (valve of Leiblein) is drawn anteriorly through the nerve ring - presumably as a result of traction exerted by the growing proboscis since there is no oesophageal loop to allow for protraction of the proboscis without affecting the oesophagus (Figs. 10 and 11).
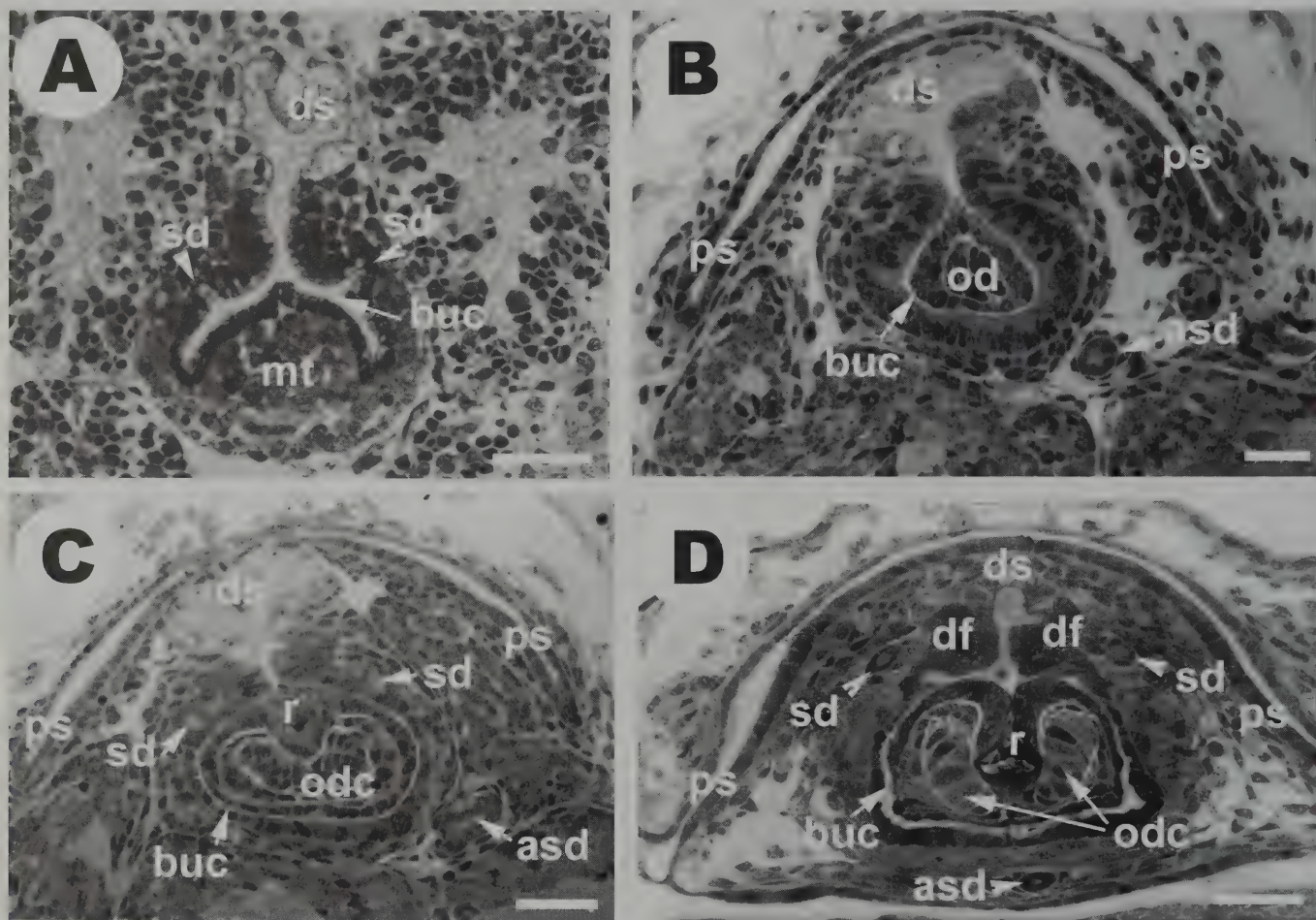
Figure 12. Buccal mass development in *Nucella lapillus* observed using light microscopy. **A.** Late stage 6, transverse section. Buccal mass lies level with nerve ring. Acinous salivary glands evaginate from buccal wall (arrowed). Scale bar 20µm. **B.** Late stage 7, transverse section - anterior portion. Buccal mass well formed, odontophore protrudes into buccal cavity, but radular does not yet wrap over anterior end. Proboscis sheath forms arc over dorsal wall of the snout. Scale bar 20µm. **C.** Late stage 7, transverse section - posterior portion. Acinous salivary gland ducts merge with wall of the buccal cavity. Single accessory salivary gland duct present. Radular protrudes into posterior portion of buccal cavity anterior to nerve ring. Proboscis sheath divided into two invaginations. Scale bar 20µm. **D.** Stage 10, transverse section. Proboscis sheath surrounds proboscis. Dorsal folds lie between buccal cavity and oesophagus. Acinous salivary gland ducts pass posteriorly ventro-lateral to dorsal folds. Radular passes into buccal cavity between odontophoral cartilages and well-developed musculature. Scale bar 20µm. (Key to lettering: asd-accessory salivary gland ducts; buc-buccal cavity; df-dorsal folds; ds-dorsal strip; mt-mesodermal tissue; od-odontophore; odc-odontophoral cartilages; ps-proboscis sheath; r-radular; sd-acinous salivary gland ducts)

By the time they hatch, the crawlaways have a long, relatively thin proboscis (Figure 10). A loop in the anterior oesophagus allows for protraction and retraction without exerting strain on the oesophagus.

Buccal mass development

In *Nucella*, the buccal cavity is derived from a pair of evaginations of the floor of the oesophagus (stage 6)(Figure 13A-B). The anterior-most cavity becomes the sub-lingual pouch, whilst the posterior cavity is the radular sac. Between the two invaginations, the floor of the oesophagus covers mesodermal tissue which later differentiates to become the odontophore and its associated musculature.

As the buccal cavity enlarges, the odontophore forms by differentiation of the underlying tissue and the musculature which operates the buccal mass forms around it and subse-
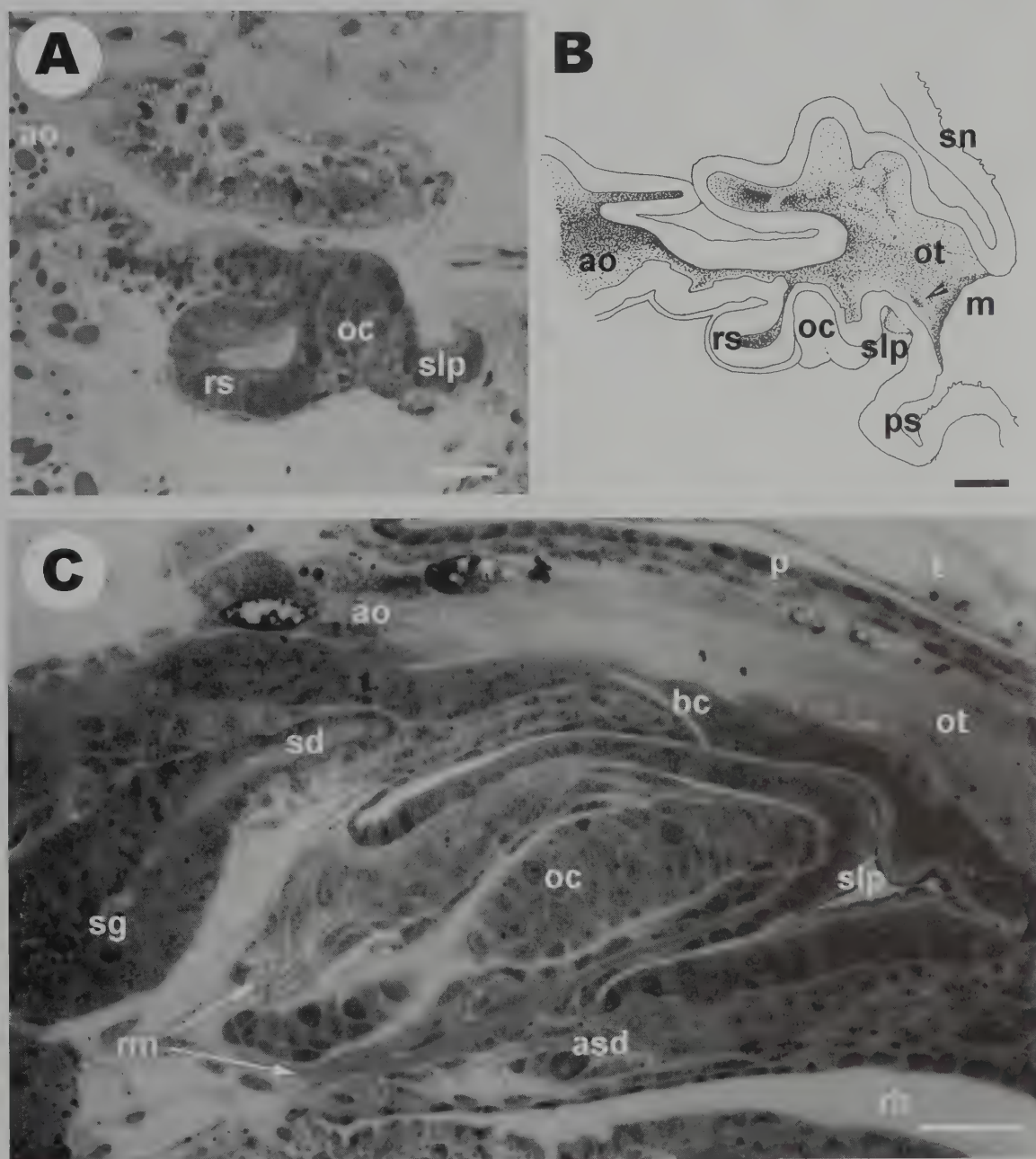
Figure 13. Buccal mass development in *Nucella lapillus* observed using light microscopy. **A.** Stage 6, longitudinal section. Radular sac and sub-lingual pouch evaginated. Odontophoral cartilages and musculature differentiate in gap between invaginations. Scale bar 20μm. **B.** Stage 6, lateral reconstruction showing the relationship between buccal cavity, mouth and oesophagus. Note first stages of ventral proboscis sheath and position of the accessory salivary gland duct on ventral lip (arrowed). Scale bar 40μm. **C.** Stage 8, longitudinal section. Opening between buccal cavity and oesophagus very narrow. Sub-lingual pouch undercuts oral tube and odontophore. Musculature highly developed. Scale bar 25μm. (Key to lettering: ao-anterior oesophagus; asd-accessory salivary gland duct; m-mouth; oc-odontophoral cartilage; ot-oral tube; p-proboscis; ps-proboscis sheath; rh-rhynchocoel; rm-odontophoral retractor muscles; rs-radular sac; sd-acinous salivary gland ducts; sg-acinous salivary glands; slp-sub-lingual pouch; sn-snout; t-tentacles)
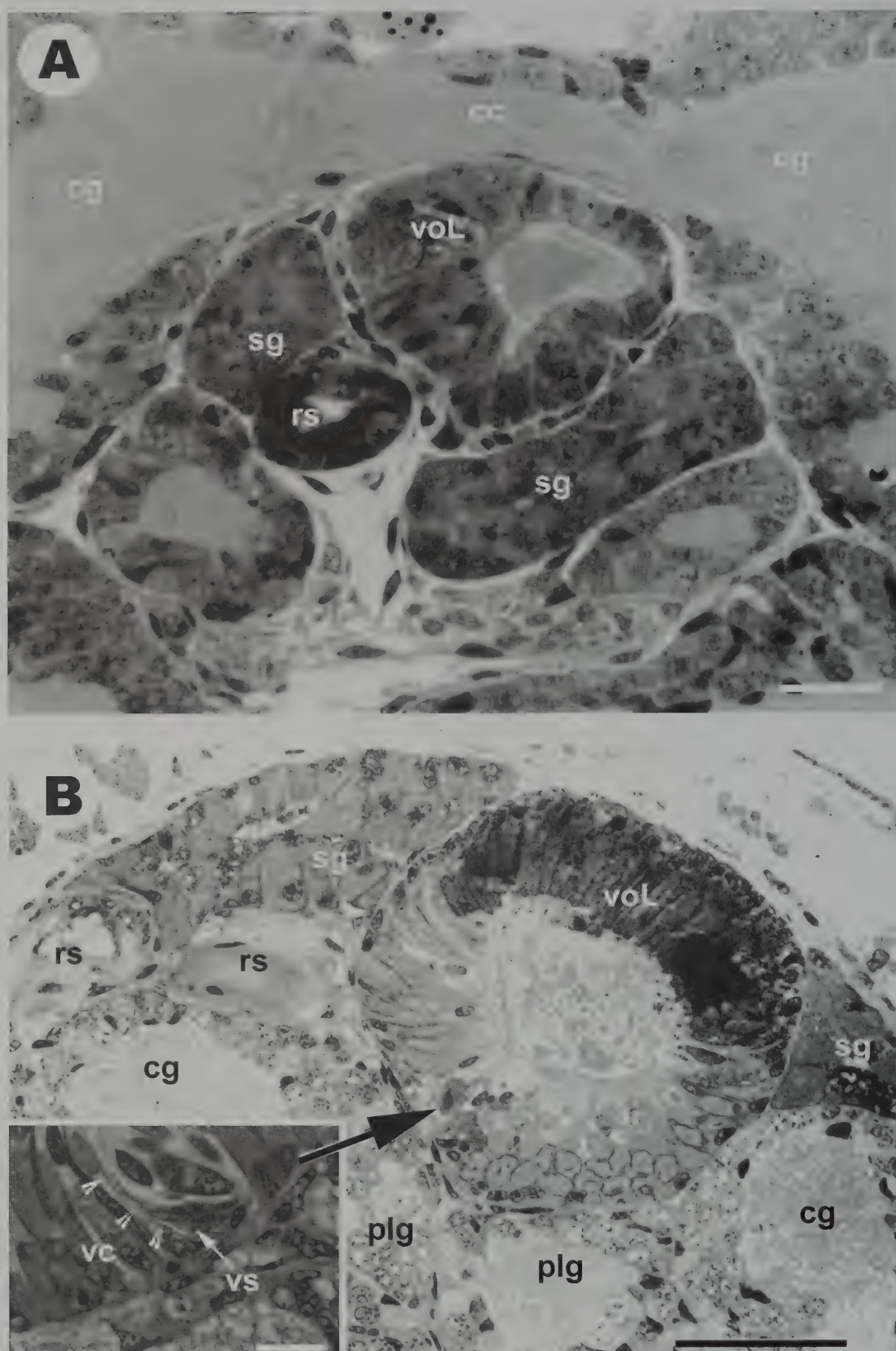
Figure 14. Relative positions of the valve of Leiblein and nerve ring in *Nucella lapillus* observed using light microscopy. **A.** Stage 7, transverse section. Valve of Leiblein, acinous salivary glands and radular sac lie within nerve ring (cg-cc-cg marks the cerebral commissure). Scale bar 10µm. **B.** Stage 10, transverse section. Valve of Leiblein, acinous salivary glands and radular sac now dorsal to nerve ring (cg-plg, plg-cg). Scale bar 25µm. Inset box shows undifferentiated ventral strip and ventral cleft (arrowed) overgrown by morphologically dorsal tissue. Scale bar 10µm. (Key to lettering: cc-cerebral commissure; cg-cerebral ganglion; rs-radular sac; plg-pleural ganglion; sg-acinous salivary glands; vc-ventral channel; voL-valve of Leiblein; vs-ventral strip)

quently becomes functional (stage 7)(Figure 11). The radular sac in *Nucella* is long, relatively thin walled and terminates in the odontoblast nest where the teeth are produced. The paired odontophoral cartilages form either side of the radular sac and as the radular is secreted by the odontoblast nest it passes into the buccal cavity and wraps over the anterior end of the odontophore (Figure 12 C-D).

The buccal mass lies within the circum-oesophageal nerve ring when it first appears and the radular sac grows posteriorly during development and initially penetrates the nerve ring (stage 7)(Figure 11C). However, during proboscis development, the buccal mass, radular sac and acinous salivary glands are all drawn anteriorly and come to lie in front of the nerve ring (stage 8). As the buccal mass moves anteriorly, the radular sac grows dorsally up the left side of the oesophagus where it is thrown into a flat spiral, dorsal to the oesophagus with the odontoblast nest at its centre (Figure 6D). During the anterior movement of the buccal mass, the radular sac is pulled through the nerve ring and consequently does not penetrate it in the adult.

Acinous salivary gland development

In *Nucella* the acinous salivary glands arise from dorso-lateral evaginations of the wall of the buccal cavity (Figure 12). They become associated with the walls of the oesophagus and lie level with the dorsal oesophageal folds, parallel to the oesophagus. At the anterior limit of the mid-oesophagus, the acinous salivary gland ducts separate from the wall of the oesophagus and the secretory regions develop, lying free in the haemocoelic cavity (Figs. 13C and 14A). During early development they grow posteriorly into the haemocoel behind the nerve ring (stages 6 and 7). As the buccal mass is drawn through the nerve ring (stage 8), the salivary glands are pulled anteriorly and come to lie wholly in the anterior part of the haemocoel. The ducts no longer pass through the nerve ring. As the glands continue to enlarge, they become dorsal to the
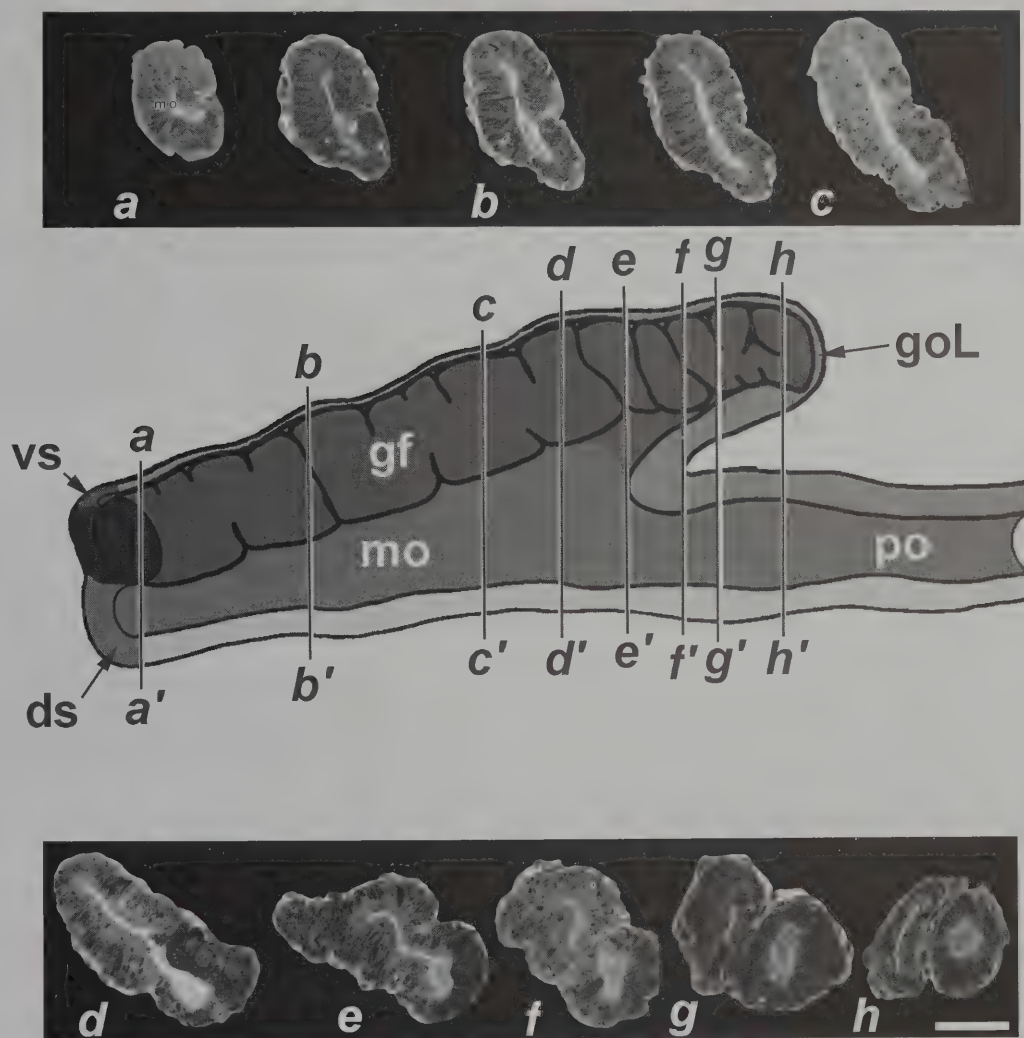
Figure 15. Reconstructions of the development of the gland of Leiblein in *Nucella lapillus* observed using light microscopy. Sections a-h transverse sections through mid-oesophagus of late stage 7 embryo at positions shown (a-a' to h-h') in diagrammatic reconstruction. Sections a-e show progressive height increase in lumen as gland of Leiblein evaginates. Sections f-h show gland of Leiblein (dorsal) and posterior oesophagus (ventral). Posterior oesophagus composed primarily of mucus secreting and ciliated cells. Scale bar 50μm. Diagrammatic reconstruction shows evagination dominated by dorsal folds and ventral strip undifferentiated. (Key to lettering: ds-dorsal strip; gf-glandular dorsal folds; goL-gland of Leiblein; mo-mid-oesophagus; po-posterior oesophagus; vs-ventral strip)

oesophagus, eventually lying above the valve of Leiblein and occupying much of the dorsal part of the anterior cephalic haemocoel (Figure 14). The radular sac passes between the two glands and the coiled radular ribbon lies above the mass of acinous secretory tissue.

Accessory salivary glands

These glands originate as a pair of ducts which arise on the ventral lip and open to the mouth (Figs. 7A and 13B). The two ducts grow posteriorly, ventral to the oral tube, and terminate just level with the buccal mass. During development, the ducts fuse along their length, first forming a single duct with paired openings to the lip and paired lumina leading to separate glands (stages 7 and 8)(Figure 7B). Finally they fuse along the length of the duct, whilst the glandular regions differentiate but remain separate. In the adult the accessory salivary glands are paired, but share a single duct which opens to the ventral lip of the mouth.

Oesophageal development

The anterior portion of the oesophagus in *Nucella* becomes differentiated relatively early in development. The oral tube consists of a simple ciliated, mucous secreting epithelium, leading from the mouth to the buccal cavity. From stage 7 onwards, the anterior oesophagus is recognisable due to the presence of ciliated dorsal folds, the ciliated, mucus secreting dorsal strip, undifferentiated ventral strip and the association between the anterior oesophagus and the acinous salivary gland ducts. The mid-oesophagus can be recognised by its relationship with the pleuro-visceral connectives and by the valve of Leiblein, glandular folds and gland of Leiblein. The valve of Leiblein becomes recognisable from stage 7 (Figure 14A). Later stages of development involve elaboration of the glandular mid-oesophageal folds (=glande framboisée) and the gland of Leiblein (from stage 8). The posterior oesophagus can only be defined once the gland of Leiblein has developed. From this point in time, the region lying posterior to the duct of the gland of Leiblein is defined as the posterior oesophagus.

Mid-oesophagus

The valve of Leiblein develops through a gradual thickening of the walls of the oesophagus, combined with differentiation to produce the ciliated oesophageal valve and the secretory mucus pad cells. The valve of Leiblein is the site of oesophageal torsion. In the adult, the mid-oesophagus is rotated through 180° relative to the anterior oesophagus. Thus the dorsal strip lies on the floor of the oesophagus and the ventral strip runs along the mid-dorsal centre-line of the roof of the oesophagus. Posterior to the valve of Leiblein, the epithelium differentiates to produce a secretory region derived from the dorsal folds. These glandular dorsal folds become very large and fill the dorsal region of the mid-oesophagus by growing over the undifferentiated ventral strip (Figure 14B, insert).

The gland of Leiblein develops at the posterior end of the mid-oesophagus and is derived from an evagination of the morphologically ventral wall of the oesophagus (Figure 15). This evagination is composed of tissue containing the undifferentiated ventral strip and the dorsal oesophageal folds. The first steps in its formation are an increase in the height of the oesophagus as the dorsal region expands, the lumen becomes taller. At the posterior limit a finger-like tubular evagination is formed which gradually enlarges and expands through later developmental steps (Figure 16).

The gland of Leiblein gradually separates from the oesophagus and a duct is formed from tissue drawn from the oesophagus anterior and posterior to the gland. The anterior face of the duct
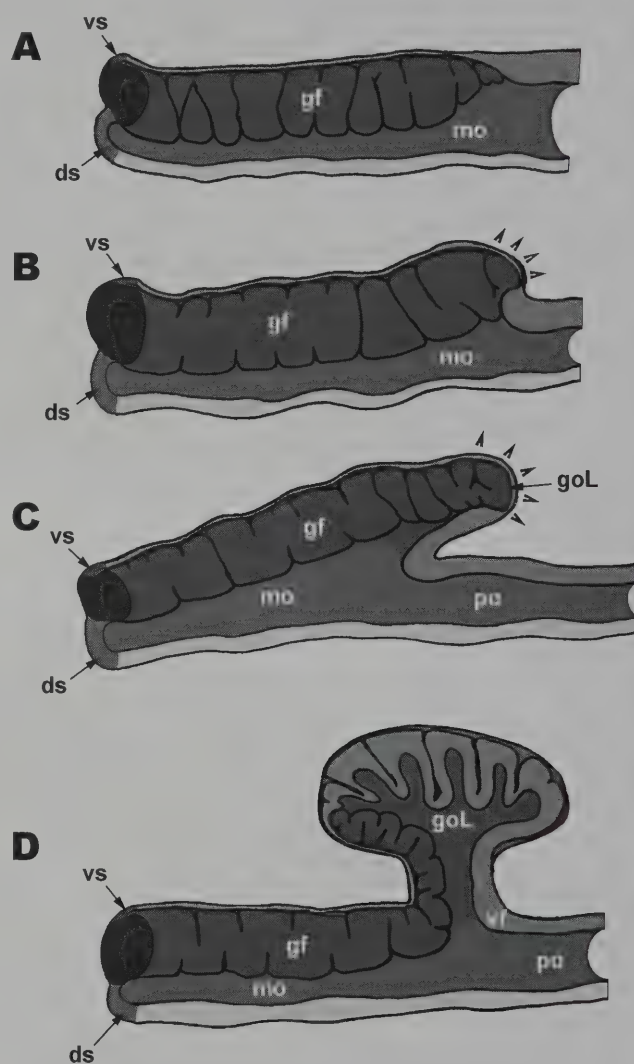
Figure 16. Development of the gland of Leiblein in *Nucella lapillus.* **A.** Differentiation of glandular folds in mid-oesophagus. **B.** Growth of glandular folds at posterior end of mid-oesophagus leads to increase in height of lumen and beginning of evagination (arrows mark growth direction). **C.** Evagination grows and outpushing develops into gland of Leiblein (arrows show growth direction). **D.** Differentiation of glandular strip produces highly folded glandular columnar epithelium, limited to anterior wall of duct and portion of floor of gland anterior to duct. Ventral strip in anterior portion of mid-oesophagus remains undifferentiated and squamous. Ventral folds differentiate and can be traced from floor of gland down posterior wall of duct into posterior oesophagus. (Key to lettering: ds-dorsal sheath; gf-glandular mid-oesophageal folds; goL-gland of Leiblein; mo-mid-oesophagus; po-posterior oesophagus; vf-ventral folds; vs-ventral strip)

bears the ventral strip flanked by the dorsal folds which form the lateral walls of the duct. The dorsal folds penetrate a short distance into the anterior portion of the gland and retain their discrete dense staining and are recognisable throughout development and into the adult. The ventral strip passes along the anterior wall of the duct, between the dorsal folds, and into the gland where it spreads and differentiates to form the glandular walls of the gland. The posterior face of the duct bears a pair of ventral folds in the adult which merge with the posterior oesophagus, but these are absent from the encapsulated developmental stages.

The gland of Leiblein is therefore morphologically ventral and rapidly becomes the most voluminous gland in the cephalic haemocoel, filling the space above the oesophagus and pressing the nerve ring and valve of Leiblein ventrally. The only other dorsally placed glands are the acinous salivary glands which lie anterior to the gland of Leiblein.
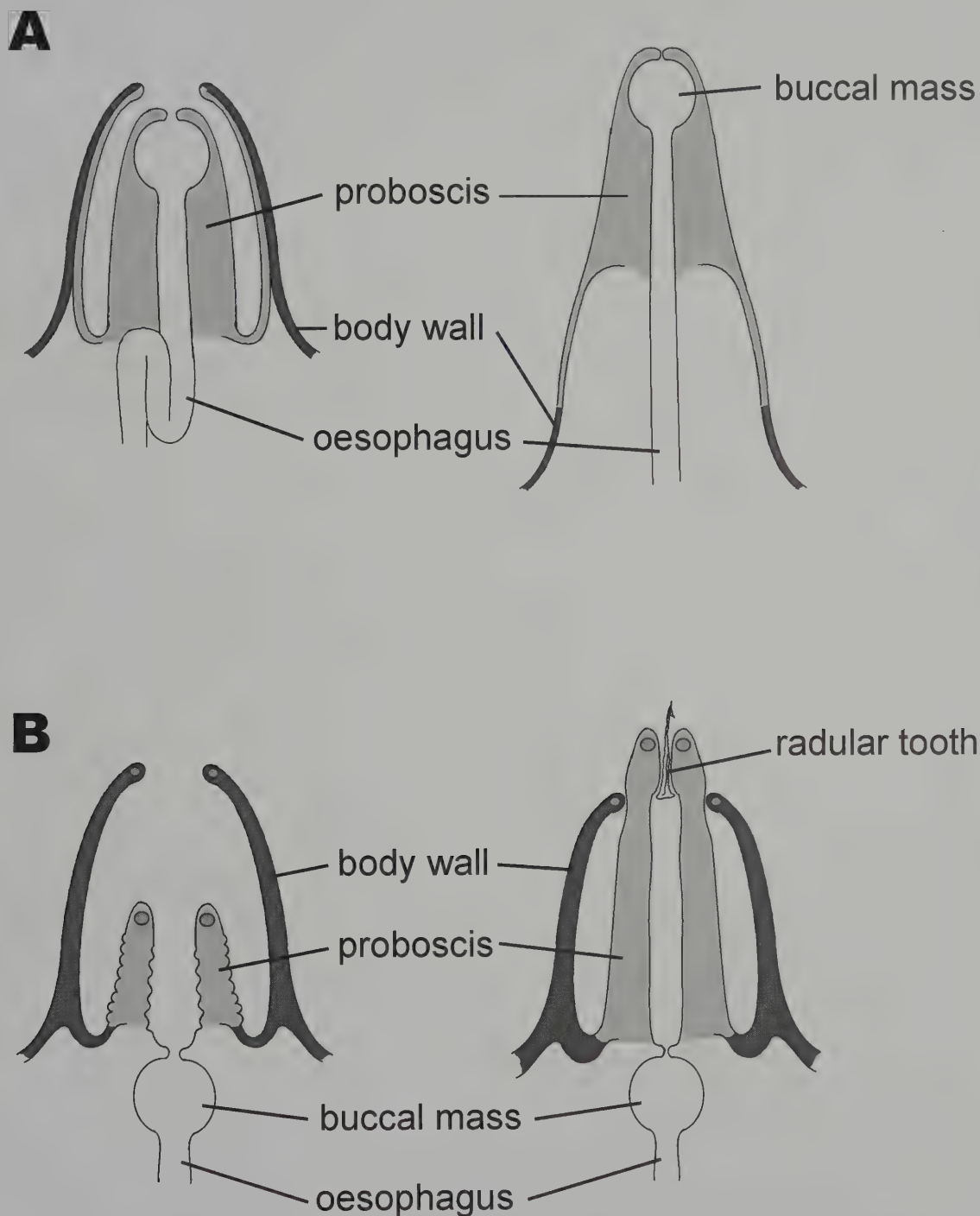
Figure 17. Proboscis types. **A.** Pleurembolic type proboscis found in *Nucella lapillus* and other Muricoidea shown in retracted and extended positions. Buccal mass is near proboscis tip and oesophagus forms a loop when proboscis is retracted. **B.** Intraembolic proboscis found in *Conus anemone* and other Conoidea. Buccal mass lies at proboscis base and extension of proboscis does not affect buccal mass or oesophagus.

DISCUSSION

Proboscis

Adult Conoidea and Muricoidea possess proboscides which have fundamental anatomical differences. In developmental terms however, both proboscis types appear to have common origins which later diverge to give radically different definitive morphologies (compare Figs. 2 and 10).

Common ontogenetic points:

- Initial growth involves the oral tube or buccal lips
- Short snout is formed prior to formation of the rhynchocoel
- Rhynchocoel is formed by paired dorsal and ventral invaginations
- All proboscis development is pre-tentacular
- Proboscis growth leads to anterior displacement of buccal mass, salivary glands and radular sac

In both species, the proboscis initially appears as an enlargement of the tissue surrounding the mouth. In *Conus anemone* the oral lips grow to form a short buccal tube which is later transformed into a short snout. In *Nucella lapillus* the initial developmental step leads directly to the formation of the snout. The presence of the velar lobes appears to be a factor in the restriction of the development of the proboscis *C. anemone* to the buccal lips rather than leading to the development of a larger
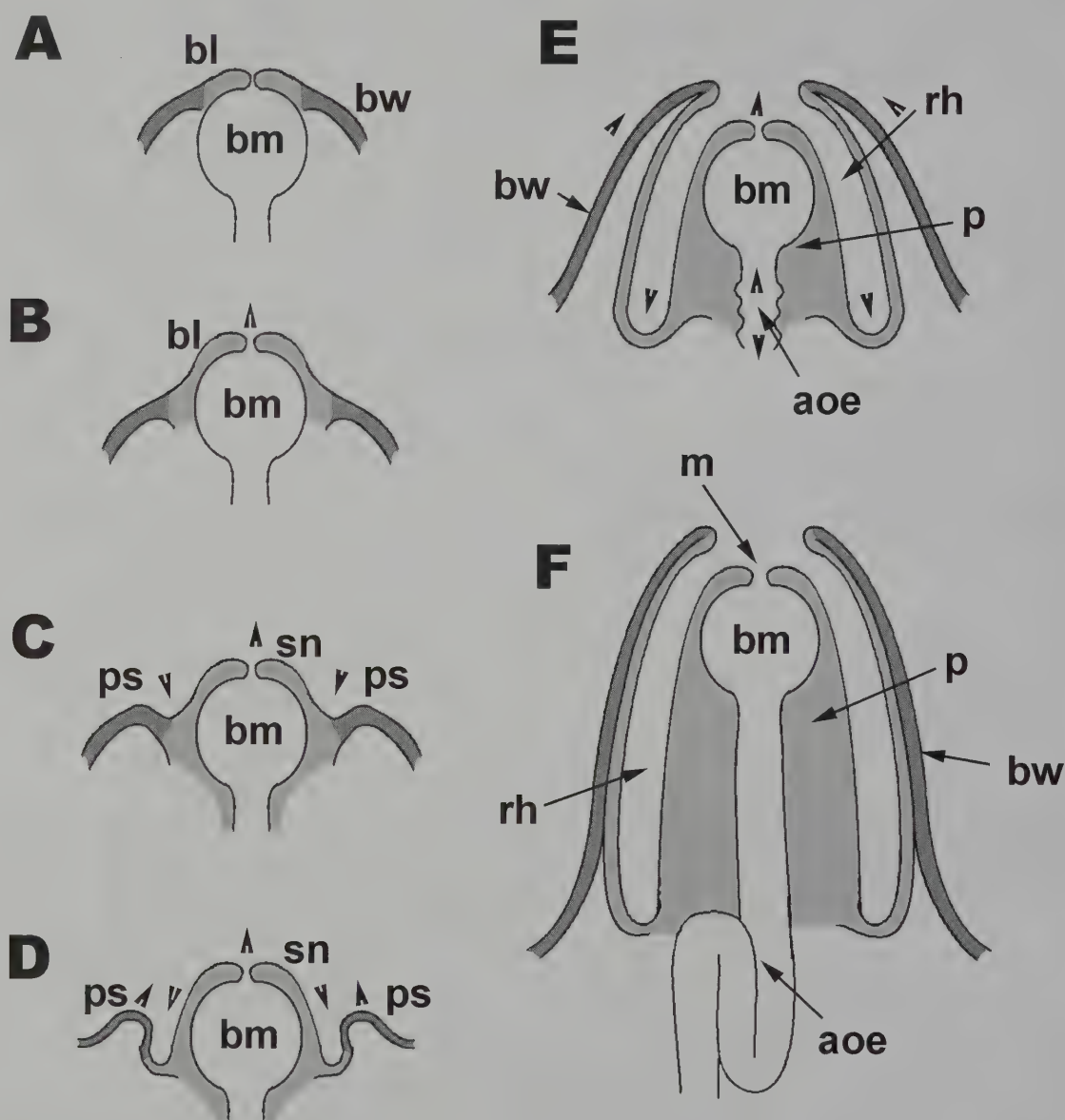
Figure 18. Proboscis development in *Nucella lapillus.* **A-C.** Stage 6, buccal lips elongate invaginations of body wall produce proboscis sheath rudiments. **D.** Stage 7. Proboscis rudiment forms buccal mass moves into proboscis. **E.** Stage 8. Proboscis elongation draws buccal mass anteriorly. Velum lost so proboscis sheath entirely surrounds proboscis. Body wall grows to cover proboscis when retracted. Folds in oesophagus allow proboscis extension, but no oesophageal loop present. **F.** Definitive state (Stage 10 onwards). Proboscis fully functional, oesophageal loop present in retracted specimens. (Key to lettering: aoe-anterior oesophagus; bm-buccal mass; bl-buccal lip; bw-buccal wall; m-mouth; p-proboscis; ps-proboscis sheath; rh-rhynchocoel; sn-snout)

snout. Velar lobes are present in *N. lapillus*, but they are very small in comparison to those of *C. anemone* (Figure 10A).
The rhynchocoel (or proboscis sheath) is a permanent cavity in adult *C. anemone*, but in *N. lapillus* the rhynchocoel is a temporary cavity formed from the proboscis wall when the proboscis is retracted. Despite these differences in the adult, the rynchocoel is derived from dorso-lateral and ventro-lateral invaginations of the body wall in both species. These invaginations eventually merge to completely surround the snout, which then begins to grow, becoming a true proboscis. In both species the dorsal and ventral invaginations do not merge with each other whilst the velar lobes are present. A possible functional cause might be because this would interrupt the food groove and prevent ciliary currents from conveying food to the mouth. This is probably irrelevant in these species which have encapsulated development, but may be significant in planktotrophic species.

The proboscis sheath is formed through anterior growth of the body wall and fusion of the tentacle bases along the dorsal mid-line (Figs. 2D-E;11; 18 and 19). This is the last stage which was observed in *C. anemone*, but in *N. lapillus*, continuation of this process of invagination and overgrowth leads to the formation of a complete sheath covering the proboscis by stage 8, by which point the proboscis becomes functional and all traces of the velar lobes have been resorbed. Proboscis development in Conoidea has never previously been described, so it is impossible to definitively state what events follow. However, it seems likely that subsequent development in *C. anemone* is similar to that of *N. lapillus* leading to the formation of a proboscis sheath formed through growth of the body wall with the rhynchocoelic cavity formed by invagination but with a proboscis derived solely from the buccal lips.

Sheridan *et al.* (1973) argued that the intraembolic conoidean proboscis type evolved from an ancestor with an acrembolic type proboscis; more recently Taylor (1986) suggested that it may equally have evolved from an ancestor with a pleurembolic type proboscis. On the basis of the common origins demonstrated in this limited study, I would suggest that the common ancestor had a proboscis type which evolved from an elongated snout, derived from the buccal lips, with a proboscis sheath derived by overgrowth of the snout rather than extensive invagination of the body wall. Thus the intraembolic type proboscis appears to be least derived. This tends to support Kantor's (1996; this volume) viewpoint that the Conoidea are basal to the Neogastropoda.

Buccal mass

Initial development of the buccal mass was not observed in *Conus anemone*, but the remainder of its ontogeny closely resembles that of *Nucella lapillus*, the key points are laid out below.

Developmental similarities:

- Radular sac initially parallel to oesophagus
- Acinous salivary glands derived from walls of buccal mass
- Radular sac rotates with respect to oesophagus
- Radular sac and acinous salivary glands penetrate nerve ring
- Buccal mass is displaced anteriorly

Key differences:

- Loss of buccal musculature in *C. anemone*
- Acinous salivary glands free in body cavity

The buccal mass in prosobranchs appears to develop via a common mechanism, through evagination of the floor of the oesophagus to form the initial buccal cavity, followed by evagination of the radular sac and then elaboration of the buccal cavity and proliferation and differentiation of the underlying mesodermal cells to form the odontophoral cartilages and buccal musculature (Figs. 12 and 13 show this process in *N. lapillus*). The initial evagination of the buccal cavity was not observed in *C. anemone* and the definitive morphology lacks both odontophoral cartilages and buccal musculature. However, the intermediate developmental steps which have been observed suggest that the origins of the buccal cavity and radular sac are probably the same as in any other prosobranch (compare Figs. 5, 12 and 13).

The development of the buccal mass in *C. anemone* follows the same pathway as that of *N. lapillus*; a ventral evagination forms the buccal cavity with a subsequent posterior evagination forming the radular sac. The radular sac grows parallel to the oesophagus until it passes posteriorly through the nerve ring, it then grows dorsally and is finally pulled anteriorly free of the nerve ring as the proboscis develops.

In *C. anemone* the buccal cavity has three distinct regions (Figure 4); the buccal sac, the radular sac and the radular caecum.

The buccal sac (which bears the ducts of the acinous salivary glands) is a thick-walled tube which separates the radular sac and radular caecum from the oesophagus (Figure 5B) and is the homologue of the dorsal part of the buccal cavity in other prosobranchs. It differs in that there are no dorsal folds passing from this part of the buccal cavity into the oesophagus, adult Conoidea appear to lack dorsal folds altogether. Its length means that it "decouples" the radular sac and sub-lingual pouch from the opening of the buccal cavity to the oesophagus. Its structure, combined with its close association with the acinous salivary gland ducts and the absence of an anterior oesophagus raises the possibility that the buccal sac may be the homologue of the anterior oesophagus. Ponder (1973) suggested that the anterior oesophagus in the Muricoidea was formed by elongation of the roof of the buccal cavity combined with closure of the ventral part. If this region were to be severely truncated, as it is in *C. anemone*, then a structure similar to the buccal sac might result. Furthermore, Kantor and Taylor (this volume) have found a valve of Leiblein in two species of Conidae, *Kermia barnadi* and *Paramontana rufozonata* (Raphitominae). In these species the venom gland opens into the buccal cavity anterior to the valve of Leiblein which is situated immediately posterior to the buccal mass. This also suggests that the anterior oesophagus has been severely truncated and restricted to the dorsal portion of the buccal mass.

Page (2000, and Page and Pederson, 1998) demonstrated that the anterior oesophagus in *Euspira lewisii* (Gould) (Neotaenioglossa: Naticoidea) and *Nassarius mendicus* (Neogastropoda: Muricoidea) is derived from the dorsal portion of the buccal cav-

ity during development. Through elongation, the buccal cavity becomes semi-isolated from the oesophagus and eventually forms a new link to the oesophagus at its anterior limit. Thus the larval mouth and adult mouth have different origins and a section of larval oesophagus dorsal to the buccal mass and anterior oesophagus is isolated and later destroyed. *E. lewisii* and *N. mendicus* both undergo planktotrophic development and it is likely that this mode of buccal mass development, first described by FRETTER (1969), enables the buccal mass to develop without interfering with the larval ability to feed on the plankton. *N. lapillus* and *C. anemone* both undergo encapsulated non-planktotrophic development and do not show this developmental pathway, but *N. lapillus* does exhibit an analogous transient narrowing of the connection between buccal cavity and oesophagus (Figs. 12B and 13C) and the opening in *C. anemone* is also narrow.

During the course of development in both *N. lapillus* and *C. anemone* anticlockwise rotation of the radular sac (when viewed from above) takes place. In *N. lapillus* the radular sac is thin walled and flexible and its rotation is less pronounced and has no effect on the walls of the buccal mass. In *C. anemone* however, the radular sac is thick walled and rigid and the buccal sac is twisted through 90° as the radular sac rotates. Whilst this is the same rotational direction as oesophageal torsion, the radular sac
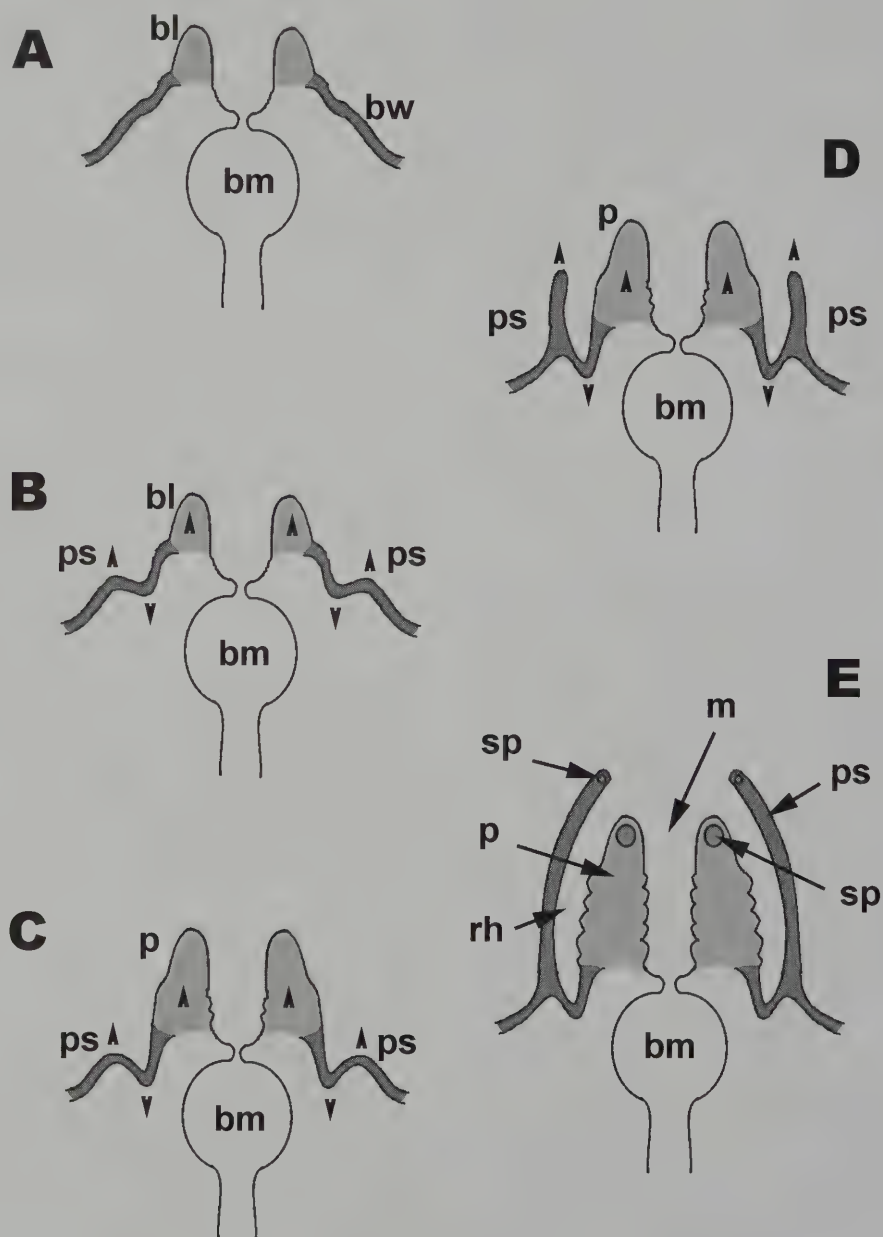
Figure 19. Proboscis development in *Conus anemone.* **A.** Stage IB. Buccal lips and oral tube elongate. **B.** Stage II. Elongated buccal lips form short tube with dorsal and ventral invaginations forming beginnings of proboscis sheath. **C-D.** Stage III. The proboscis begins to elongate and is overgrown by body wall and tentacle bases to form partial proboscis sheath. **E.** Definitive state. Proboscis lies within rhynchocoel. (Key to lettering: bm-buccal mass; bl-buccal lips; bw-body wall; m-mouth; p-proboscis; ps-proboscis sheath; rh-rhynchocoel; sp-sphincter muscles)

lies perpendicular to the oesophagus and the buccal mass lies anterior to the point of torsion in *N. lapillus* (the point of torsion in *C. anemone* has not been described), so the rotation is presumably not due to the buccal mass being dragged in one direction leading to the radular sac adopting the opposite orientation. At present the significance (if any) of this observation is unclear.

The radular caecum forms from the anterior limit of the ventral buccal cavity and is probably the homologue of the sub-lingual pouch. In other prosobranchs this would be the point where worn teeth would break free of the radular ribbon. In the Conoidea it is the site where detached radular teeth are stored prior to their utilisation for envenomation.

Both *N. lapillus* and *C. anemone* show anterior displacement of the buccal mass during development which seems to be linked with the elongation of the snout to form the proboscis. Its effects are less pronounced in *C. anemone* than in *N. lapillus* since most proboscis growth occurs anterior to the buccal mass. Alternatively, reorganisation of the foregut may be a normal developmental process in Neotaenioglossa which has received little attention up to this point. Certainly some adult neotaenioglossans show anteriorly displaced acinous salivary glands (HOUBRICK 1980; 1984; 1993; TAYLOR and MORRIS 1988) and an anterior movement of the buccal mass during development was reported in the *Lacuna vincta* (Neotaenioglossa) (which has no proboscis) and *Nassarius incrassatus* and *Nassarius reticulatus* (Neogastropoda: Muricoidea) by ABRO (1969) and FRETTER (1969).

This process would therefore seem to be connected with the elongation of the anterior portion of the head to form a snout or proboscis and is less conspicuous in species where this elongation is less pronounced.

The buccal mass in *N. lapillus* comprises a complex internal and external musculature. This appears to be involved in determining the definitive position of the buccal mass within the proboscis to some extent in *N. lapillus*. In adult *C. anemone* the buccal walls are muscular, but there are no odontophoral cartilages and none of the associated musculature. However, early in the development of *C. anemone* there are muscles associated with the buccal mass (Figure 5D). An anterior muscle linking the anterior part of the sub-lingual pouch to the body wall near to the mouth is probably a velar retractor muscle. These have been shown to form the median protractor muscles of the sub-radular membrane in other neogastropods (GRAHAM, 1973). Perhaps more significant, is the presence of a posterior muscle which passes from the postero-ventral face of the buccal mass to the pedal musculature. This is absent from the adult *C. anemone*, but the development of a muscle with the same insertion and origins can be observed in *N. lapillus* where it forms the odontophoral retractor muscle (Figs. 12B and 13C). In both species this muscle passes through the nerve ring. The role of the buccal musculature in positioning the buccal mass in *C. anemone* is uncertain, since late proboscis development was not observed. However, the loss of the buccal musculature in *C. anemone* is probably a derived feature since it appears to begin to develop ventral to the radular sac in a developmental stage analogous to *N. lapillus* stage 6, (stage II) but later degenerates.

The basal buccal mass found in the Conoidea has been described by KANTOR (1996; this volume) as a primitive feature. KANTOR suggests that a hypothetical neogastropod archetype might have a basal buccal mass and a short proboscis and that the neogastropods diversified from this point prior to the evolution of the various proboscis types now present in the order. A few other neogastropods are known to have a basal buccal mass; *Benthobia tryoni* (Pseudolividae) and *Olivella borealis* (Olivellidae) were described by KANTOR (1991; 1996). KANTOR (1991; 1996; this volume) suggested that the possession of radular retractor muscles which pass through the nerve ring, as seen in some species of the turrid subfamily Drillinae, in *Benthobia* and in most lower caenogastropods is also a primitive feature which is absent from neogastropods with an elongate pleurembolic proboscis. The radular retractor muscles pass through the nerve ring in the muricids *N. lapillus* (Ball, 1994) and *Urosalpinx cinerea* (Carriker, 1943) which have relatively short proboscides. However, in the Buccinidae, where the proboscis is much longer, this muscle originates in the proboscis wall anterior to the nerve ring (GRAHAM, 1973). The presence of these muscles in a transient form during the development of *C. anemone* suggests that this arrangement may well be a conserved feature.

Acinous and accessory salivary glands

The acinous salivary glands in *C. anemone* and *N. lapillus* have the same derivation and follow an almost identical pattern of development (Figs. 5 and 11). The key differences are that the ducts are free in *C. anemone* and do not become associated with the walls of the oesophagus. In *N. lapillus* both ducts of the acinous salivary glands penetrate the nerve ring early in development whilst in *C. anemone*, the twisting of the buccal sac means that only a single gland penetrates the nerve ring whilst the other grows anteriorly (Figure 5). Elongation of the proboscis causes the salivary glands come to lie in front of the nerve ring so that the ducts no longer penetrate the nerve ring.

The accessory salivary glands in both species arise as paired ducts on the ventral lip of the mouth (Figure 7). In *C. anemone* these paired ducts appear to fuse early in development and only a single duct is present in the adult. In *N. lapillus* the ducts have been shown to fuse progressively during development so that a single duct leads to a pair of glands (BALL, 1994; BALL *et al.* 1997b) (Figure 7B).

The common origins and developmental pattern of acinous and accessory salivary glands in *C. anemone* and *N. lapillus* is further proof of the homology of these glands within the Neogastropoda. Furthermore, it can be seen from this study that the adult state of the glands does not reflect the whole story and this could be of importance when interpreting character states and in determining whether organs have been lost or secondarily regained.

Mid-oesophageal gland

In *C. anemone* the mid-oesophageal gland is composed of a long, coiled tubular duct (the venom gland) terminating in a muscular bulb (Figure 4). In *N. lapillus* the mid-oesophageal

gland consists of a bulbous gland (the gland of Leiblein) connected to the oesophagus by a short duct. PONDER (1970) speculated that the venom gland in cones is homologous with the glandular mid-oesophageal folds (=glande framboisée, *sensu* AMAUDRUT, 1898) of the Muricoidea and that the conoidean muscular bulb is the homologue of the gland of Leiblein. In *N. lapillus* the glandular mid-oesophageal folds are derived directly through differentiation of the dorsal folds whilst the gland of Leiblein develops from the morphologically ventral strip. The gland of Leiblein is therefore derived from tissue which is predominantly morphologically ventral in origin and the morphologically dorsal glandular folds do not contribute to the secretory portion of the gland, although they are present in part of the duct in the adult (Figure 16).

In the developing *C. anemone* embryos a blunt, finger-like outpushing at the posterior limit of the oesophagus forms during stage III (Figure 9). This is composed predominantly of the glandular ventral tissue and might represent the first stage in the development of the venom apparatus. However, since no subsequent developmental stages could be examined, later events are still unknown. The only author to have described *Conus* development (Franc, 1943) simply stated (translated from the original French - p.122) that "in the larvae of *C. mediterraneus*, [the venom apparatus] is composed of, as in the adult, an elongated bulb, placed against the stomach at the posterior part of the oesophagus, whose anterior extremity extends via a long and contorted tube, which, later, occupies a large part of the free space in the cephalic cavity." His illustration of the developing venom gland (FRANC, 1943; Figure 73, page 122), shows a cross section of the duct with a diameter of approximately 50µm composed of perhaps a dozen secretory cells with basal nuclei and apical secretory granules. These cells have the same appearance as secretory cells found in the ventral mid-oesophagus of *C. anemone* (Figure 8). FRANC (1943 - p.123) found that the venom apparatus in *C. mediterraneus* was "clearly differentiated by the time of hatching", but he did not describe the appearance of the larvae at various developmental stages and does not show or discuss the development of any other part of the foregut or its glands.

The similarity of the cells illustrated by FRANC (1943) and those of the out-pushing in *C. anemone* suggests that the evagination, which starts close to the mouth and terminates near the posterior limit of the secretory portion of the oesophagus, is the origin of the venom gland. The muscular bulb presumably forms at the termination of the out-pushing, perhaps by proliferation and differentiation of the mesodermal cells which form a thin coat over the length of the oesophagus (Figure 9).

Although it is disappointing to be unable to examine the final stages in development, the possibility that the venom gland forms through evagination in a manner similar to the formation of the muricid gland of Leiblein is further evidence for the homology of this gland throughout the Neogastropoda. Furthermore the ventral origins of the secretory cells of this putative venom gland rule out any possibility of homology between the conoidean venom gland and the muricoidean glandular dorsal folds.

Competence of hatchlings

N. lapillus hatches as miniature, sexually immature adults which possess all of the definitive foregut features together with a functional proboscis and radula. Examination of bivalve shells kept with emergent crawlaways shows that many of them were drilled. GOSSELIN and CHIA (1994) also showed that *Nucella emarginata* crawlaways were able to feed three days after hatching.

C. anemone emerges at the pediveliger stage. The hatchlings have a large foot and crawl actively, but the velar lobes are still present, albeit shrunken in some individuals and the animals are no longer able to swim. At this stage the acinous and accessory salivary glands are well formed, but proboscis and venom gland development are incomplete, this would suggest that they would be unable to feed. However, in one larval specimen a radular tooth was found at the mouth suggesting that teeth can be detached and transported from the radular sac by this stage in development (Figure 3). Thus the emergent *C. anemone* larvae may not have a fully functional complement of foregut glands, but may nevertheless still be able to envenomate prey. Neither can the possible use of accessory salivary gland secretions be ruled out. However, they probably suffer limitations in their prey handling ability due to the lack of a fully formed proboscis and this probably means that the juvenile and adult diets are necessarily different, although this could only be confirmed by behavioural studies.

CONCLUSIONS

In general *Conus anemone* follows a similar developmental pattern to *Nucella lapillus*. This suggests that the autapomorphic features of the neogastropod foregut are homologous in both superfamilies and supports the monophyly of the Neogastropoda.

Retardation in the developmental process, perhaps due to the presence of redundant planktotrophic feeding structures, lack of true adaptation to encapsulated development (lack of food eggs) and limited protolecith reserves may explain the comparatively early developmental stage at which *C. anemone* hatches from the egg capsule.

Detailed examination of the post-hatching stages of *C. anemone* development, either in *C. anemone* or another suitable species, is necessary to determine the latter stages of proboscis and venom apparatus development. However, it is apparent that many of the specialised features of the conoidean foregut have common origins with the less specialised muricid foregut.

Heterochrony has been suggested as an evolutionary mechanism for generating morphological diversity amongst the Neogastropoda (BALL, 1994). It is clear that there is considerable plasticity in the neogastropod foregut as evidenced by the high diversity in foregut types (see TAYLOR, KANTOR and SYSOEV, 1993; KANTOR, Medinskaya and TAYLOR, 1997, for example). Changes in the rate of development in certain key organ systems could be responsible for major differences in adult morphology.

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Biometrics of *Nassarius mutabilis* (l.) (Gastropoda, Nassaridae) in the Central Adriatic Sea

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KEY WORDS: Neogastropoda, commercial species, Nassaridae, *Nassarius mutabilis*, Adriatic Sea.

ABSTRACT Shell height, shell diameter, total weight and fresh meat weight were measured on specimens of *Nassarius mutabilis* (L.) collected with a dredge at two different depths in the central Adriatic sea. The results obtained separately for the two sexes showed that females generally have a higher shell height and that, starting from a certain size, they tend to become more globular and heavier than males.

RIASSUNTO Sono stati analizzati altezza e diametro della conchiglia, peso totale e peso fresco della carne su esemplari di *Nassarius mutabilis* (L.) catturati tramite campionamenti con draga effettuati a due diverse profondità nel medio Adriatico. I risultati relativi ai due sessi evidenziano che le femmine mostrano una taglia maggiore e che, a partire da una certa dimensione, tendono anche ad assumere una conformazione più globosa e un peso, sia totale che della carne, superiore rispetto ai maschi.

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INTRODUCTION

Nassarius mutabilis (L.) inhabits fine sands and fine muddy sands at depths between 2 and 15 m along Mediterranean coasts (FISCHER *et al.*, 1987). It is a gonochoristic species which reproduces from the end of winter to the beginning of spring. Fertilization is internal and eggs are layed between march and may in conical, transparent egg capsules, which attach to hard substrates (FABI & GIANNINI, 1983). Indirect development results in the production of a veliger (RIEDL, 1991). *N. mutabilis*, as all other members of the family, is a carnivore which feeds on dead organisms, although it may predate live animals, suffocating them by means of its foot (TORELLI, 1982). It spends most of the day buried in the substrate with its proboscis protruding from it and emerges during the night when it wonders around in search of food, like his congeneric species (BEDULLI, 1976; CRISP, 1978).

N. mutabilis is especially abundant in the central Adriatic sea, where it represents an important resource for small-scale fisheries (PICCINETTI & PICCINETTI-MANFRIN, 1998). Fishing for this species takes place between the beginning of autumn and the end of spring, by use of a special baskets, like traps, baited with dead fish.

Unlike other members of the same genus (e.g., *Nassarius reticulatus* L.), knowledge on the biology of *N. mutabilis* is poor, despite its commercial importance. This study is, therefore, aimed at expanding the knowledge on the biology and biometry of this gastropod.

MATERIALS AND METHODS

Seasonal sampling (one for each season) was carried out off Senigallia, along the Italian coasts of the central Adriatic sea between October 1988 (autumn) and June 1989 (summer), at two different depths (6 and 13 m) by use of a modified dredge containing a 6 mm mesh bag. All specimens of *N. mutabilis* were preserved in alcohol (90°). The height (H) of each individual was measured and its sex determined by visual analysis of external reproductive organs. The few suspect imposex individuals were discarded. Size-frequency distributions for the two sexes were obtained from these data. One-way analysis of variance (ANOVA; LINDMAN, 1992) was used to compare mean size of males and females at different depths and seasons. Height data were log-transformed (log$x$) in order to satisfy normality and homoscedasticity requirements. Normality was tested using a Chi-square goodness-of-fit test and homoscedasticity by use of Bartlett's test. Both tests resulted positive in all cases with the exception of data from 13 m depth, which resulted heteroscedastic and unsuitable for the application of ANOVA.

Maximum diameter of the shell (D), total fresh weight (Wt) and fresh weight of the meat (Wm) were measured for a subsample of individuals from each season. Regression equations for H/D, H/Wt, and H/Wm were calculated separately for males and females. Outliers were identified using Cook's test and eliminated. The regression coefficients obtained were compared between sexes using a t-test.

RESULTS

The size-frequency distributions show that, at all depths and in all seasons, females had a greater size than males. Such difference resulted significant ($p<0.01$) in all cases with the sole exception of the 6 m depth ($p=0.895$), which was the only case where both sexes were prevalently represented by small individuals (males: mean $H=13.9\pm3.0$ mm; females: mean $H=14.1\pm3.9$ mm). A great discrepancy was found in the abundance of *N. mutabilis* at 6 m and 13 m in spring, the latter being approximately four times the former (fig. 1; Tab. I).

Table II summarises the regression equations obtained for H/D, H/Wt and H/Wm for each sex (258 females and 205 males). In all cases a significant difference was scored in the

regression coefficients of males and females.

Males of the smaller size classes had slightly greater diameter, total weight and meat weight than females with equal lengths. With increasing size this difference decreased and, eventually, reversed. In fact, from 11.5 mm and 14.5 mm in height, Wt and Wm respectively, were greater in females than in males. This trend became increasingly obvious as animals got larger. For example: at H = 5 mm, Wt and Wm were 0.45 g and 0.012, respectively in males and 0.42 g and 0.009 g, respectively in females. On the contrary, at H = 25 mm, Wt and Wm were 3.04 g and 1.19 g, respectively in males and 3.23 g and 1.33 g, respectively in females.

CONCLUSIONS

The size-distributions obtained for *N. mutabilis* show that, in agreement with data reported for other Neogastropods (Fretter, 1984), males generally have a smaller mean size than females. Furthermore, height/diameter and height/meat weight ratios show that, starting from 14.5 mm, corresponding to approximately one year of age (Cespuglio *et al.*, 1999), the shells of females gain a more globose shape compared to males. This is associated to a greater increase in meat weight in females. Total weight, too, shows an increase in females with respect to males, starting from 11.5 mm. Such differences become increasingly evident as size increases, as already observed in other prosobranchs (Fretter, 1984). Nevertheless, the differences between male and female *N. mutabilis* are not marked enough to allow inference of true systematic sexual dimorphism.

Discrepancies in the mean size of individuals found at 6 m and 13 m, lead to the conclusion that there may be a difference in spatial distribution according to size, smaller individuals being more frequent in shallow waters and larger ones, at greater depths.

Finally, the difference in abundance between the two depths in spring, where significantly lower numbers of individuals were recorded at 6 m, may be correlated with the fact that *N. mutabilis* reproduces in this period (Fabi & Giannini, 1983; Cespuglio *et al.*, 1999). Mass migrations are, in fact, quite common in the Nassaridae during this phase of their biological cycle (Tallmark, 1980; Lambeck, 1984).

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Table I. Medium sizes (Hm) ± standard deviation and total number of specimens (Ntot) obtained for males and females of *N. mutabilis* at the two dephts examinated (6 and 13 m) in all seasons.

Season:	6 m		13 m	
	males	females	males	females
Autumn	Ntot = 160 Hm = 18.3±2.2 mm	Ntot = 61 Hm = 22.5±2.4 mm	Ntot = 137 Hm = 20.3±2.8 mm	Ntot = 66 Hm = 23.2±2.3 mm
Winter	Ntot = 98 Hm = 13.9±3.0 mm	Ntot = 129 Hm = 14.1±3.9 mm	Ntot = 49 Hm = 18.6±2.3 mm	Ntot = 79 Hm = 23.2±3.6 mm
Spring	Ntot = 28 Hm = 19.6±2.8 mm	Ntot = 34 Hm = 22.9±4.4 mm	Ntot = 151 Hm = 18.5±2.1 mm	Ntot = 103 Hm = 23.5±2.7 mm
Summer	Ntot = 51 Hm = 19.6±2.3 mm	Ntot = 109 Hm = 21.0±3.1 mm	Ntot = 64 Hm = 18.7±2.4 mm	Ntot = 237 Hm = 19.9±3.6 mm

Table II. Regression equations obtained for H/D, H/Wt and H/Wm for 205 males and 258 females of *N. mutabilis.*

Relation	Sex	N	Equation	R^2	p
H/W_t	Males	205	$Wt = 0.0007 * H^{2.6134}$	0.970	<0,01
	Females	258	$Wt = 0.0006 * H^{2.6888}$	0.970	
H/W_m	Males	205	$Wm = 0.0001 * H^{2.8829}$	0.892	<0,01
	Females	258	$Wm = 0.0001 * H^{3.0676}$	0.896	
H/D	Males	205	$D = 0.723 * H$	0.823	<0,01
	Females	258	$D = - 0.751 + 0.776 * H$	0.897	
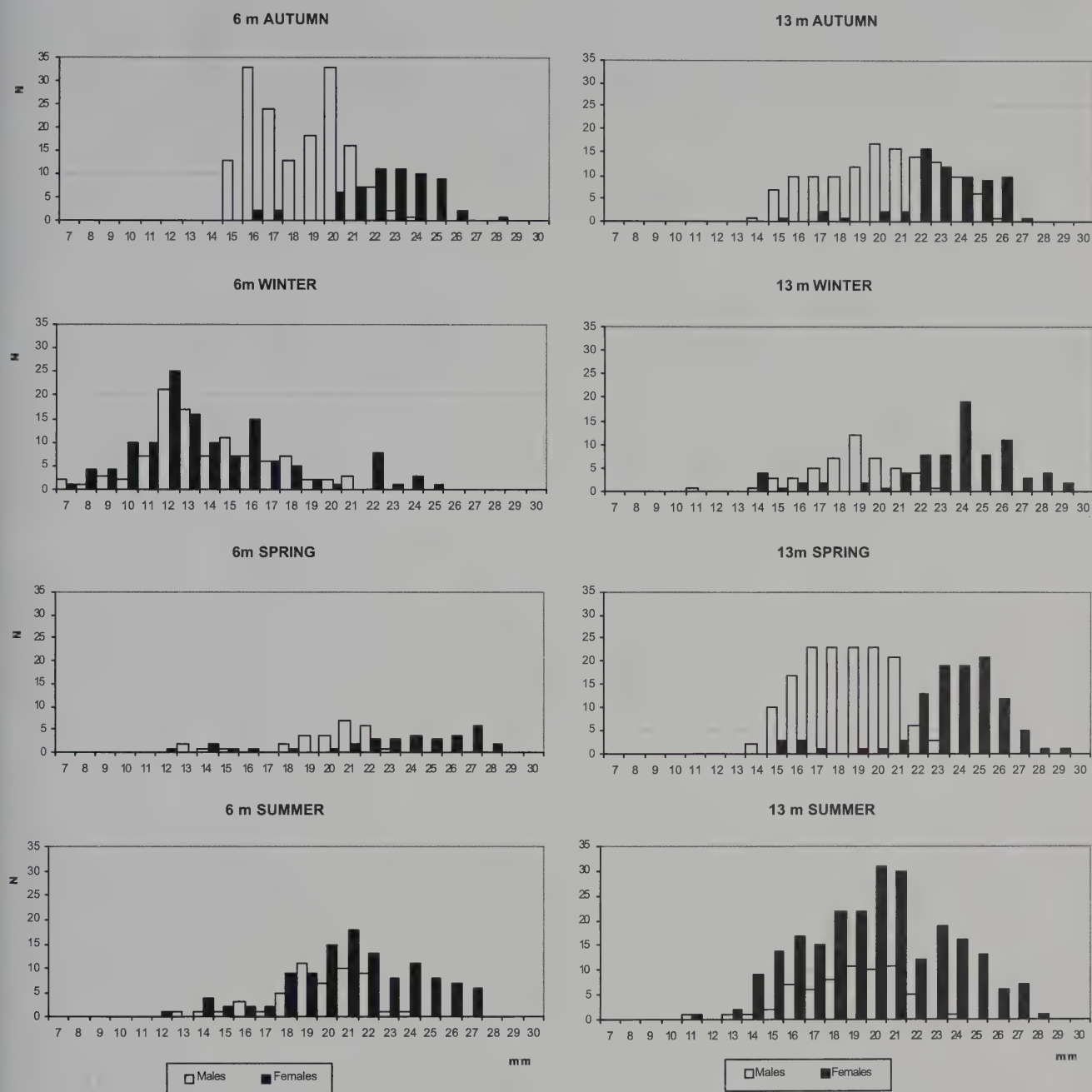
Figure 1. Size-frequency distributions obtained separately for males and females of *N. mutabilis* at the two depths examined (6 m and 13 m) in all seasons.

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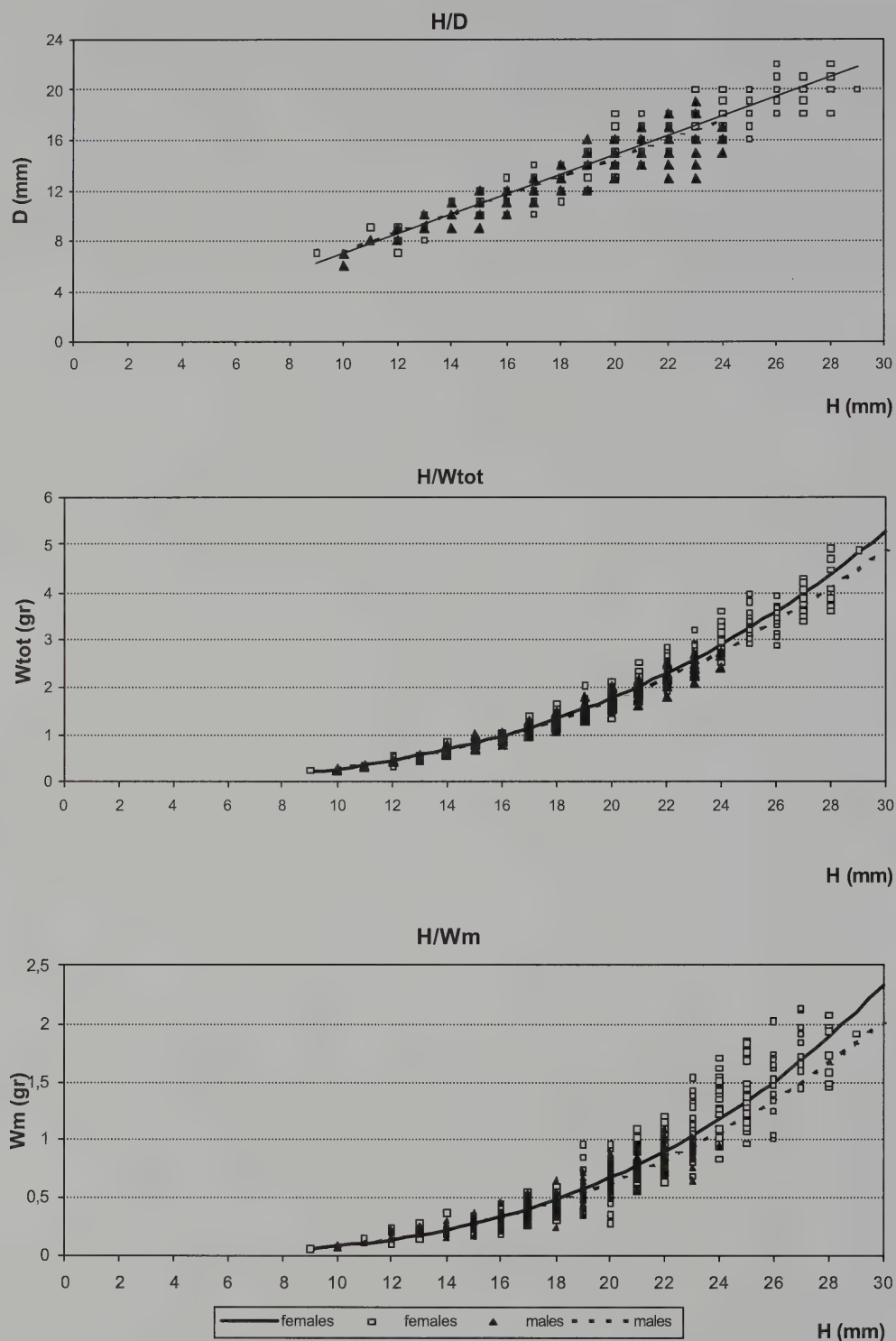
Figure 2. Regression relations obtained for H/D, H/Wt and H/Wm for 205 males and 258 females of *N. mutabilis*.

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Foregut anatomy and relationships of raphitomine gastropods (Gastropoda: Conoidea: Raphitominae)

Yuri I. Kantor & John D. Taylor

KEY WORDS: Conoidea, Raphitominae, anatomy, foregut, feeding mechanism, phylogeny.

ABSTRACT The Raphitominae (formerly Daphnellinae) are, from shell characters, probably the most morphologically disparate of the conoidean subfamilies, including both some of the smallest and largest species of the superfamily. A study by serial sections of 14 species revealed considerable variation in the configuration of the foregut. Species differ in the presence, position and morphology of the major structures such as proboscis, buccal mass, septum, glands and sphincters of the buccal tube. Distinctive features of raphitomines, although not found in every species, are a rhynchostomal introvert, a rhynchodeal septum, the needle-like radular teeth and the muscular bulb consisting of a single muscle layer. In *Kermia barnardi* and *Paramontana rufozonata* there is a valve situated just posterior to the buccal cavity resembling the valve of Leiblein of Rachiglossa and Nematoglossa A remarkable feature of the Raphitominae is the independent reduction and loss of major foregut organs - the proboscis may be long, reduced, vestigial or totally absent. The radula, salivary glands and venom apparatus may be present or absent. Usually radula loss is correlated with the loss of the venom gland but in *Pseudodaphnella granocostata* the venom apparatus persists but the radula is absent. *Teretiopsis* species lack a proboscis, radula, venom apparatus or salivary glands. Three main types of feeding are proposed for the Raphitominae: 1) normal toxoglossan feeding with use of teeth at the proboscis tip for stabbing and envenomation of prey. 2) envenomation of the prey without stabbing by radular teeth. 3) capture of prey without stabbing and envenomation, probably by suctorial means. Raphitomines have the most disparate foregut configurations of any conoidean subfamily. The rhynchostomal introvert is otherwise found only in Terebridae and the rhynchodeal introvert found in many terebrids and some *Conus* species. Tubular salivary glands are also found in Mangeliinae and some Crassispirinae. Phylogenetic analysis suggests that the Raphitominae have closest similarity with the Coninae and Mangeliinae.

RIASSUNTO Le Raphitominae (precedentemente note come Daphnellinae) sono, dal punto di vista conchiliare, la più disparata delle sottofamiglie di conoidei, comprendendo alcune tra le più piccole e le più grandi specie della superfamiglia. Uno studio condotto con sezioni seriali su 14 specie ha rivelato una variabilità considerevole nella configurazione del canale alimentare anteriore. Le specie differiscono nella presenza, la posizione e la morfologia delle maggiori strutture come la proboscide, la massa boccale, il setto, le ghiandole e gli sfinteri del tubo boccale. Caratteristiche distintive delle Raphitominae, anche se non riscontrabili in tutte le specie, sono un introverto rincostomale, i denti radulari aghiformi ed il bulbo muscolare consistente in un singolo strato muscolare. In *Kermia barnardi* e *Paramontana rufozonata* c'è una struttura situata proprio posteriormente alla massa boccale somigliante alla "valve of Leiblein" di Rachiglossa e Nematoglossa.Una caratteristica rimarchevole delle Raphitominae è la riduzione e/o la perdita indipendente dei maggiori organi del canale alimentare anteriore – la proboscide può quindi essere lunga, ridotta, vestigiale o totalmente assente. La radula, le ghiandole salivari e l'apparato velenifero possono essere presenti o assenti. Normalmente la perdita della radula è correlata con la perdita della ghiandola del veleno, ma in *Pseudodaphnella granocostata*, l'apparato velenifero persiste pur essendo la radula assente. Specie di *Teretiopsis* mancano della proboscide, della radula, dell'apparato velenifero o delle ghiandole salivari. Tre tipi principali di modalità alimentari sono proposte per le Raphitominae: 1) normale alimentazione toxoglossa con uso dei denti radulari all'apice della proboscide per colpire ed iniettare il veleno nella preda; 2) uso dell'apparato velenifero ma senza colpire con la radula; 3) cattura della preda senza radula ne uso del veleno, probabilmente per attività suttoria. Le Raphitominae possiedono anche la più disparata serie di configurazioni del canale alimentare anteriore di tutte le sottofamiglie di conoidei. L'introverto rincostomale si ritrova altrove solo tra le Terebridae e l'introverto rincodeale si trova in molte terebre e in alcune specie di *Conus*. Le ghiandole salivari sono presenti anche nelle Mangeliinae e in alcune Crassispirinae. Un'analisi filogenetica suggerisce che le Raphitominae hanno stretta similarità con le Coninae e le Mangeliinae.

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INTRODUCTION

Gastropods of the superfamily Conoidea are notable for the possession of a large, coiled venom gland, together with highly modified radular teeth used to inject venom into the prey. Although *Conus* is the most well known taxon (KOHN, 1990; OLIVERA *et al.* 1990), it represents only a small part of the total diversity of the group. Classifications have largely been based on shell and radular characters (POWELL, 1966; MCLEAN, 1971) but recent studies are providing anatomical criteria, mainly derived from characters of the foregut, for the definition of suprageneric taxa of conoideans (TAYLOR, KANTOR & SYSOEV, 1993; KANTOR, MEDINSKAYA & TAYLOR, 1977; MEDINSKAYA, 1999). Amongst the species studied so far a wide disparity in the configuration of the various organs of the foregut has been revealed and new arrangements are continually being discovered. Preliminary studies of the diverse subfamily Raphitominae (formerly Daphnellinae) suggested a wide variation in foregut anatomy, including the apparent loss of major structures (SMITH, 1967a: SHERIDAN, BOUILLON & VAN MOL, 1973; TAYLOR *et al.*, 1993). For this reason we decided to investigate the anatomy of the Raphitominae in more detail.

The Raphitominae is probably one of the most species-rich of all the conoidean subfamilies, exceeded only by the Mangeliinae. Fifty-seven Recent genera and subgenera are listed by TAYLOR *et al.* (1993). Raphitomines have a world-wide distribution and inhabit a wide range of habitats from the
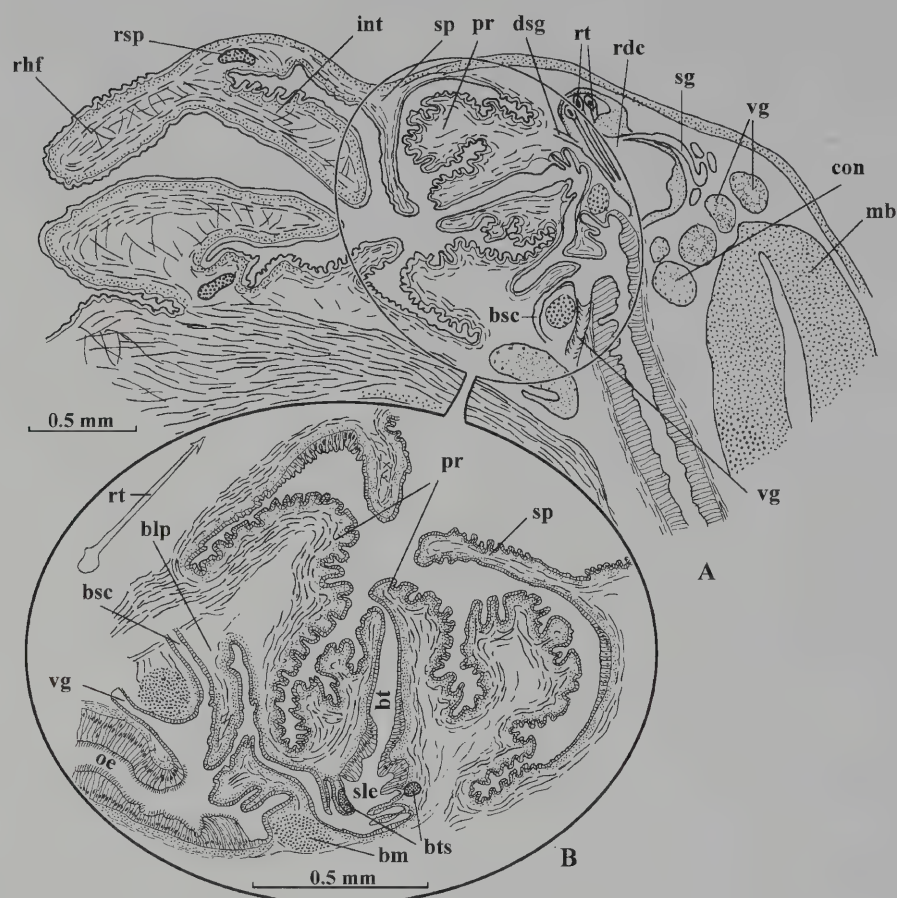
Figure 1. *Gymnobela pyrrhogramma* (Dautzenberg & Fischer, 1896). A. Semidiagrammatic longitudinal section of the foregut. B. Enlarged region of the proboscis and buccal mass (the single marginal tooth is drawn in the same scale to the left of the proboscis).
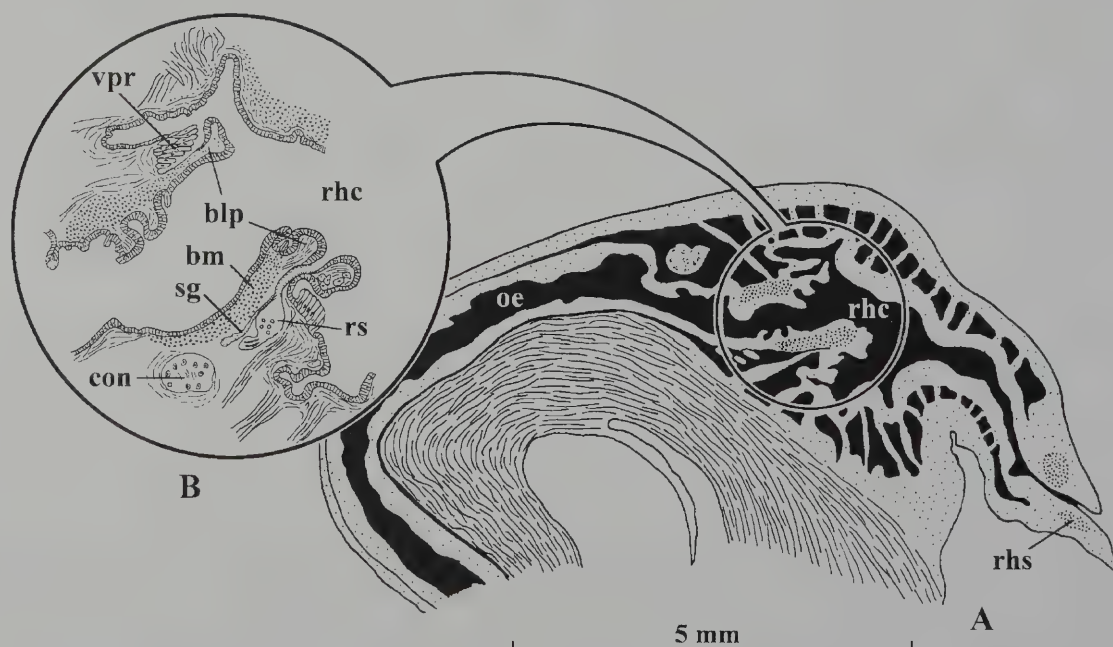
Figure 2. *Gymnobela emertoni* (Verrill & Smith, 1884) [after Taylor *et al.*, 1993, slightly modified]. A. Semidiagrammatic longitudinal section of the foregut (rhynchocoel — solid black). B. Enlarged region of the proboscis and buccal.

intertidal boulders of coral reefs (KAY, 1990) to abyssal depths (BOUCHET & WARÈN, 1980) including hydrothermal vents (SYSOEV & KANTOR 1995; BECK, 1996). The group encompasses some of both the smallest and largest conoideans ranging from around 1.5 mm to 136 mm shell height (POWELL, 1966). Notable are some of the large deeper water taxa, such as *Pontiothauma, Spergo* and *Thatcheria*. Apart from brief accounts of anatomy and habitat little is known about the biology of any species. Raphitomines also have a rich fossil record in the Cenozoic, PACAUD & LE RENARD (1995) for instance, record 80 species from the Palaeogene of the Paris Basin.

Despite the wide disparity in shell form Raphitominae are usually recognised by two shell characters, namely, the diagonally cancellate larval shell and the shape of the posterior apertural canal which is located at the suture in the form of reversed L shape (POWELL, 1966; MCLEAN, 1971). These characters are, however, not present in all species. Raphitomines also lack an operculum. Many small species of conoideans currently classified in the Mangeliinae and other subfamilies are very poorly known and on investigation some of these have been shown to possess both raphitomine shell and anatomical characters as exemplified by recent studies of *Clathromangelia* (OLIVERIO, 1995) and *Hemilienardia* (TAYLOR *et al.* 1993 and herein). For *Philbertia* and *Caenodagreutes*, SMITH (1967b) showed how species with very similar shells possess very different internal anatomies.

Previous anatomical studies of a few raphitomine species have indicated a puzzling disparity of foregut anatomy (KANTOR & SYSOEV, 1986; 1989; OLIVERIO, 1995; PACE, 1903; SMITH, 1967a: SHERIDAN *et al.*, 1973; SYSOEV & KANTOR 1995; TAYLOR, *et al.* 1993). Some species possess a full range of conoidean foregut organs including the venom apparatus, salivary glands, proboscis and radula whilst in others some or all these structures are absent. Additionally, some species have been described with structures such as the rhynchodeal introvert and septum which had otherwise been found only in species of Terebridae. Initial phylogenetic analysis identified the Raphitominae along with the Taraninae as the most derived groups of conoideans (TAYLOR *et al.* 1993), however, it was uncertain whether the Raphitominae constituted a monophyletic group.

The objectives of this study were to establish the morphological range and disparity of the foregut in Raphitominae, to reappraise previous descriptions of raphitomine anatomy, and to use an analysis of foregut characters to explore relationships both within family and with other conoideans.

MATERIAL AND METHODS

Details of the species studied are listed in Table 1. For all species, longitudinal serial sections were made of the foregut, cut at 8-10 µm and mostly stained in Masson's trichrome. Radulae were cleaned in a dilute sodium hypochlorite solution, washed in distilled water, and air dried onto circular glass coverslips. These were then glued to stubs, sputter coated and then examined by SEM.

Abbreviations used on anatomical figures:
asg - accessory salivary gland
bc - buccal cavity
blp -buccal lip
bm - buccal mass
bsc - buccal sac
bt - buccal tube
"btc" - cylinder of the buccal tube
bts - buccal tube sphincters
cf - circular fold
cm - columellar muscle
cmf - circular muscle fold
con - circumoesophageal nerve ring
dasg - duct of the accessory salivary gland
dsg – duct of salivary gland
dvg - duct of the venom gland
epp - epithelial pad;
glc - glandular cells
glf – glandular fold
dvg - duct of venom gland
int - introvert
mb - muscular bulb of the venom gland
oe – oesophagus
pr – proboscis
prr – proboscis retractors
rdc - radular caecum
rhc - rhynchocoel (rhynchodeal cavity)
rhf - rhynchostomal funnel
rns - rhynchostome
rs - radular sac
rsp - rhynchostomal sphincter
rt - radular tooth
sg -salivary gland
sle - sac-like enlargement of the buccal tube
sng – snout gland
sp - septum
tm - transverse muscles
vg - venom gland
vl - valve
vpr - vestigial proboscis

SPECIES DESCRIPTIONS

In this section we describe and illustrate the foregut anatomy and radulae of each of the species examined. Nomenclature of organs and structures largely follows TAYLOR *et al.* (1993) and KANTOR *et al.* (1997). Although we have studied only a small proportion of the living genera and species, our coverage includes gastropods of very different sizes and from widely different habitats, ranging from the shallow intertidal to abyssal depths and hydrothermal vents.

Gymnobela pyrrhogramma (Dautzenberg & Fischer, 1896)
(Figures 1, 3 A-B, 20A)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is small and located posteriorly
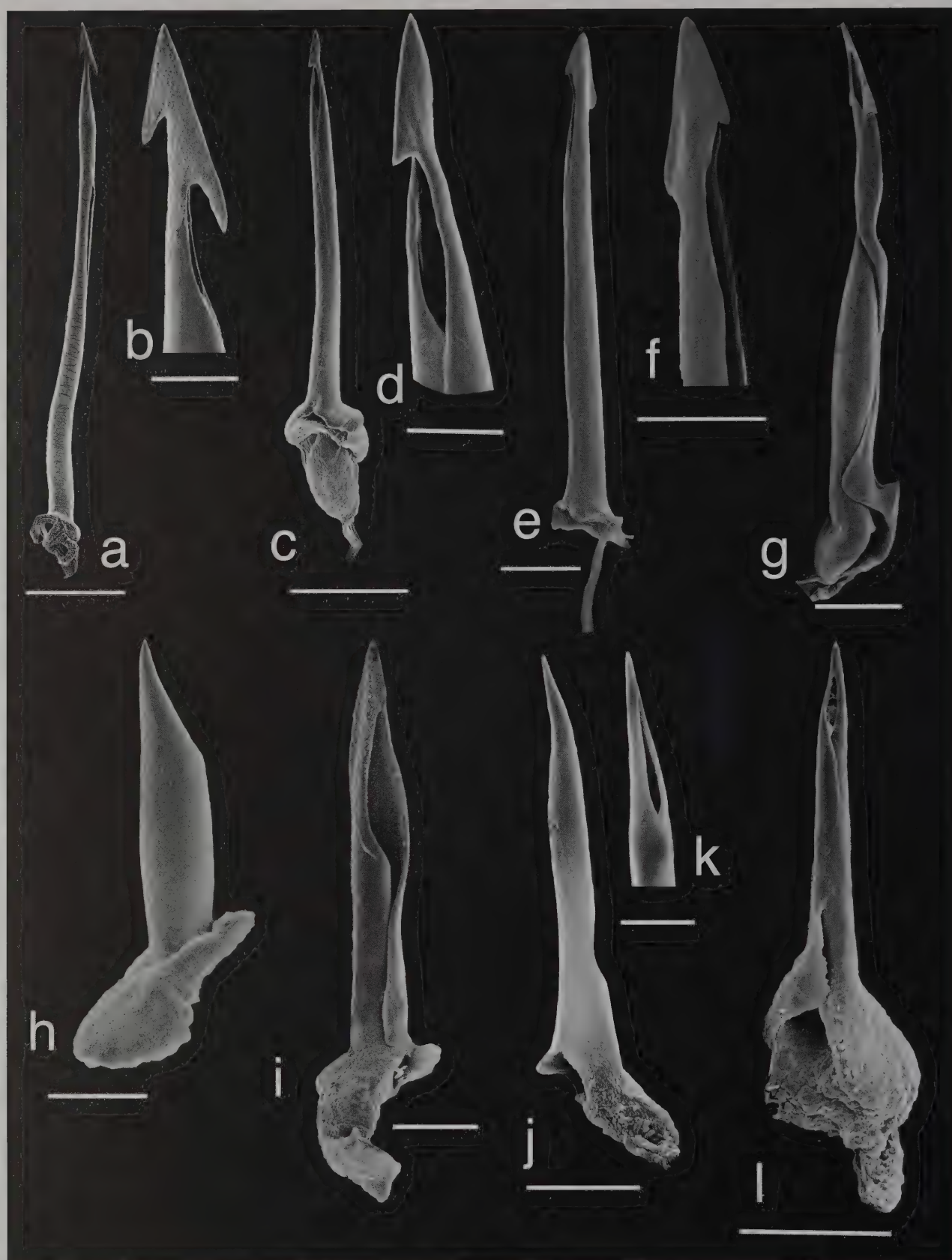
Figure 3. Marginal radular teeth of Raphitominae and *Conus boholensis*. **A.** *Gymnobela pyrrogramma.* Scale bar = 100µm . **B.** detail of tip of A. Scale bar = 20µm. **C.** *Phymorhynchus moscalevi.* Scale bar = 50µm. **D.** detail of tip of C. Scale bar = 10µm . **E.** *Pontiothauma mirabile.* Scale bar = 50µm. **F.** detail of tip of E. Scale bar = 50 µm. **G.** *Conus boholensis.* Scale bar = 100µm. **H.** *Hemilienardia malleti.* Scale bar = 20µm . **I.** *Thatcheria mirabilis.* Scale bar = 50µm. **J.** *Paramontana rufozonata.* Scale bar = 10 µm. **K.** Detail of tip of H. Scale bar = 5µm. **L.** *Gymnobela emertoni.* Scale bar = 20 µm.

within the rhynchostome. There is large rhynchostomal introvert which lacks a sphincter at the tip. The introvert when withdrawn occupies slightly more that half the length of the rhynchocoel. The epithelium of the body wall is cuticularized and composed of low, columnar cells. These are replaced by tall, columnar, ciliated cells at the proximal end of the retracted introvert and then by a tall, columnar, cuticularized epithelium near the introvert tip. Thus, when everted the introvert would have a ciliated epithelium, while its inner surface would be cuticularized. A thin but muscular, rhynchodeal septum with a narrow orifice divides the rhynchocoel medianly.

The proboscis, when retracted, is very short, and lies posterior to the septum. Its walls form numerous, large, circular folds, which indicate a potential for great extension of proboscis length on protraction. The mouth is very narrow. Powerful proboscis retractor muscles run along the proboscis walls. The buccal tube forms numerous long circular folds at the base of the proboscis, which probably straighten when the proboscis is protracted. Within the buccal tube there is a prominent, sac-like enlargement at some distance behind the mouth opening. This is lined by a tall columnar epithelium which forms a pad. A small anterior sphincter lies at the base of this sac-like enlargement. The distance from the mouth opening to the sphincter is about equal to the length of a radular tooth and therefore this sphincter is able to hold the base of a tooth. The buccal tube is lined with a very low epithelium, bearing short cilia, and has relatively thick walls only slightly thinner than those of the proboscis.

Buccal mass and oesophagus

The buccal mass is very short, with thin walls, and a possible sphincter just at the junction with the oesophagus. There are medium-sized, extensible buccal lips which may be inverted posteriorly into the buccal cavity. The oesophagus is wide and lined with a tall ciliated epithelium with glandular cells.

Table 1. Details of Raphitominae specimens sectioned for analysis.

Gymnobela emertoni (Verrill & Smith, 1884). 4706 m, North Atlantic, Biogas station CP17. 46°31' N, 10°20' W. The Natural History Museum, London.

Gymnobela pyrrhogramma (Dautzenberg & Fischer, 1896). 590 m, N. Atlantic, BIACORES station 161, 37°40' N, 25°51' W. The Natural History Museum, London.

Gymnobela sp. unnamed. see Kantor & Sysoev. 1996. 3610 m, E. Tasman Sea, 44°18' S, 166°46' E, Galathea station 607, Zoological Museum of University of Copenhagen, uncatalogued.

Teretia teres (Forbes, 1844), 250 m Söreidsvik, Norway. coll. J.D. George, 1973. The Natural History Museum, London.

Teretiopsis abyssalis Kantor & Sysoev, 1989. Sectioned holotype, 5510 m, 39°57' N, 165°07' E, (E. of Japan), R/V Vityaz stn 3156, Zoological Museum of Moscow State University, Moscow, Lc-5680.

Teretiopsis levicarinatus Kantor & Sysoev, 1989. Sectioned holotype, 2800m, 5°02' N, 20°50' W off Liberia, Zoological Museum of Moscow State University, Moscow, Lc-5679.

Kermia barnardi (Brazier, 1876). Intertidal rocks, Pointe Ouen Toro, Near Nouméa, New Caledonia. Coll. J.D. Taylor, 1989. The Natural History Museum, London.

Hemidaphne reeveana (Deshayes, 1863). Intertidal reef edge, Asan Bay, Guam. Coll. J.D. Taylor 1986. The Natural History Museum, London.

Hemilienardia malleti (Récluz, 1852). Intertidal reef edge, Asan Bay, Guam. Coll. J.D. Taylor 1986. The Natural History Museum, London.

Pseudodaphnella granicostata (Reeve, 1846). Intertidal reef edge, Asan Bay, Guam coll. J.D. Taylor 1986. The Natural History Museum, London.

Paramontana rufozonata (Angas, 1877). Intertidal rocks, Cape Vlamingh, Rottnest Island, Western Australia. Coll J.D. Taylor 1996. The Natural History Museum, London.

Thatcheria mirabilis Angas, 1877. 440 m, W. of Lacepede Archipelago, Western Australia, 16°54' S, 119°52' E. The Natural History Museum, London.

Phymorhynchus moscalevi Sysoev & Kantor, 1995. Sectioned paratype, 3680m, Mid-Atlantic Ridge, 26°08' N, 44°49' W, Zoological Museum of Moscow State University, Moscow, Lc-22458.

Phymorhynchus wareni Sysoev & Kantor, 1995. Sectioned paratype, 1483m. Edison Seamount, S. of Lihir Island, W. Pacific, 3°18.85' S, 152°34.9' E, Canadian Museum of Nature, Ottawa, CMN 92955.

Pontiothauma mirabile Smith 1895. 2540m, Indian Ocean, 6°59' N, 78°50' E SAFARI Stn. CP5. Radula only. The Natural History Museum, London.

Also

Conus boholensis Petuch, 1979. New Caledonia, Musèum national d'histoire naturelle, Paris.

Glands

The salivary glands are paired, tubular, long and highly convoluted, although very narrow, measuring about 30 µm in diameter. The epithelium, lining the glands comprises uniform, tall, columnar, ciliated cells. The ducts are not differentiated from the glands and open into proximal part of the radular caecum.

The venom gland does not change in histology after passing anteriorly through the nerve ring and opens ventrally into the posterior part of the buccal mass. The muscular bulb is large, long and oval, with the wall formed from a single thick layer of circular muscle fibres. The lumen of the bulb is narrow and lined with a low, non-glandular epithelium.

Radula

The radular sac is large and long and situated dorsally to the oesophagus (only the radular caecum is shown on Figure (1A). The radular caecum is medium sized. The buccal sac (i.e. that portion of the radular sac lying between the entrance of the salivary ducts and the buccal cavity) is long and narrow (Fig. 1B - bsc). It passes downward along the right side of the buccal mass, and then gradually turns towards its narrow ventral opening into the buccal cavity just to the anterior of the of the buccal mass "sphincter". The radular teeth (Fig. 3 A-B) are hollow, long (630 µm), slender, slightly curved and with an enlarged base. The distal tip of the tooth has two distinct barbs with the adapical opening located behind the second barb.

Gymnobela emertoni (Verrill & Smith, 1884)
(Figures 2, 3L, 20B)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is medium-sized and anteriorly located. Rhynchostomal introvert absent. There is no rhynchodeal septum present. There are well-developed transverse muscles connecting the rhynchodaeum with the body walls. The proboscis is vestigial and represented by a low circular fold with an epithelium of tall, columnar cells.

Buccal mass and oesophagus

The buccal mass is small and thin-walled. There are small buccal lips, which are longer,than the proboscis itself. The buccal tube is absent due to the near complete reduction of the proboscis.

Glands

The unpaired salivary gland is very small, tubular and straight. The venom gland is absent.

Radula

The radula sac is very short and contains very few teeth. The buccal sac opens ventrally near the tip of the buccal lip. The
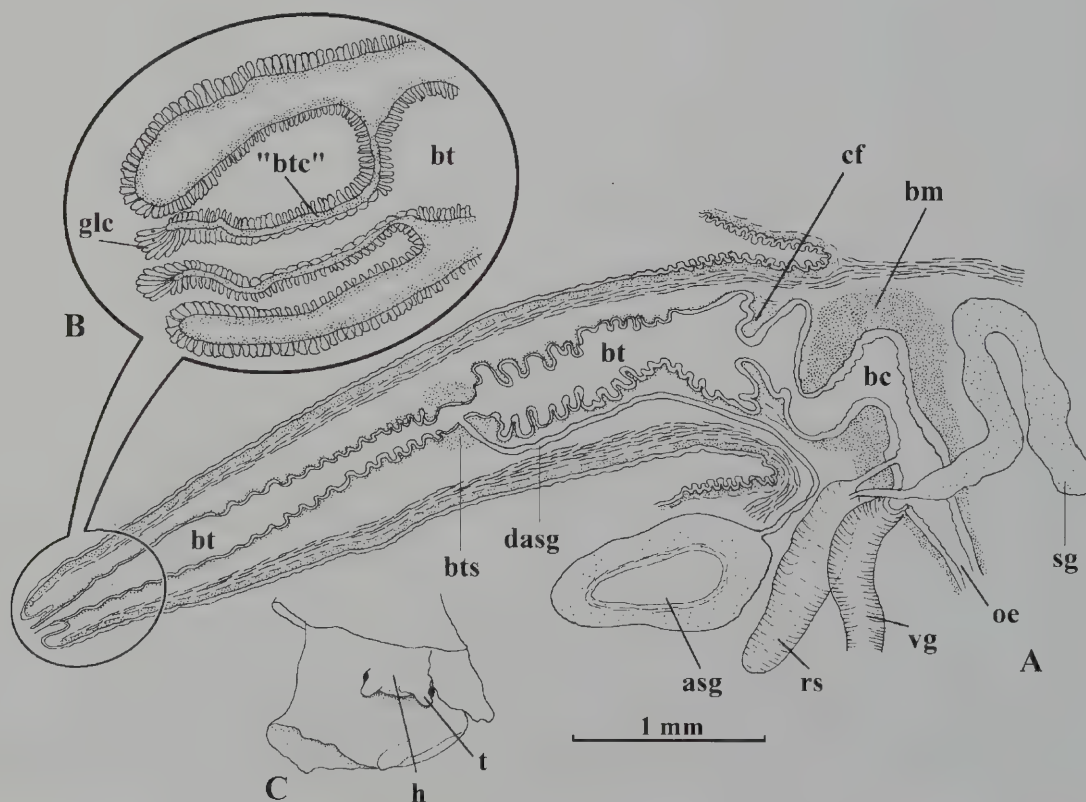
Figure 4. *Gymnobela* sp. [after Kantor, Sysoev, 1996, slightly modified]. **A** . Semidiagrammatic longitudinal section through the proboscis and buccal mass region; nervous ring not shown. **B**. Enlarged proboscis tip. **C**. Head.

radular teeth (Fig. 3L) are 60-70 μm long, enrolled, simple, awl shaped with pointed tip and a large club-shaped base.

Gymnobela **sp.**
(Figures 4, 20C)

This is an undescribed species, provisionally assigned to *Gymnobela* on the basis of shell characters, which possesses an unusual anatomy with the description modified from Kantor & Sysoev (1996). Only the anterior part of the digestive system was serially sectioned after removal from the body haemocoel.

Rhynchodaeum and proboscis

Rhynchostomal introvert absent. There is no rhynchodeal septum present. The proboscis is very long, folded several times within the rhynchodeal cavity and rather broad at the base (in the figure the proboscis is shown straight and somewhat shorter). The proboscis is thin-walled and lined with tall, goblet-shaped cells, which are cuticularized in the posterior part of the proboscis. Powerful proboscis retractor muscles run along the proboscis walls. The border between the muscles and the wall is unclear so that retractors give the appearance of a thick proboscis wall. The wall of anterior part of the proboscis forms an invagination, into which the anterior part of the buccal tube protrudes like a cylinder (Figure 4B "btc"). The opening of the buccal tube, which should be considered as the mouth, is very small. The epithelium lining the anteriormost part of the buccal tube is formed by tall, elongated, probably glandular cells (Figure 4B - glc). The buccal tube is rather thick-walled, and lined with a low epithelium. The wall of the buccal tube is composed of a layer of connective tissue, underlain by a layer of circular muscle fibres. A large sphincter is located approximately mid-way along the length of the proboscis. At the proboscis base the buccal tube forms a low circular fold (Figure 4A - cf).

Buccal mass and oesophagus

The large, oval, buccal mass is situated at the base of the proboscis. The walls of the buccal cavity are moderately thin and formed by circular muscle fibres.

Glands

A single, convoluted, tubular, salivary gland opens through a moderately long duct into the anterior part of the radular sac. There is rather large accessory salivary gland, which is oval with broad lumen. The inner layer of epithelium is very thin and comprises no more than 1/5 of the thickness of the gland wall. The layer of muscle fibres is also very thin. The duct of the gland opens into the buccal tube mid-way along the length of the proboscis near to the buccal tube sphincter.

The venom gland is well developed, of uniform histology, long, convoluted, and opens into the posterior part of the buccal cavity close to the opening of the radular sac. The large, oval, muscular bulb is formed by an outer, very thin layer of longitudinal muscle fibres, a layer of connective tissue and thick, inner layer of longitudinal muscle fibres.

Radula

A small radular sac opens into the right side of the posterior part of the buccal cavity. The radular sac is thin-walled, without a pronounced caecum. No radular teeth were present in the sac.

Pseudodaphnella granicostata **(Reeve, 1846)**
(Figures 5, 20M)

Rhynchodaeum and proboscis

The rhynchostomal introvert absent. The rhynchostomal sphincter is medium-sized and located rather to the posterior of the rhynchostome. Anterior to the sphincter there is a large, fold of circular muscles (Fig.5A - cmf). The rhynchostomal lips are poorly muscular and extend to form a rhynchostomal funnel. The epithelium of the anterior part of the rhynchocoel between the rhynchostome and the sphincter bears long cilia, while in the sphincter area the epithelium is cuticularized and similar to that lining the rest of the rhynchocoel. The epithelium of the rhynchodaeum is folded. In the posteriormost part of rhynchocoel there is low septum, (fig. 5B - sp), the epithelium behind this changing to tall, columnar cells.

The proboscis is very reduced, short, and represented only by a circular fold, through which long and muscular buccal lips are protruded.

Buccal mass and oesophagus

The epithelium lining the anterior third of the buccal lips externally and the buccal mass and oesophagus bears very long cilia (9-15 μm). The buccal mass is not differentiated from the buccal lips and extends to the base of the proboscis. The buccal tube is absent because of the reduction of the proboscis.
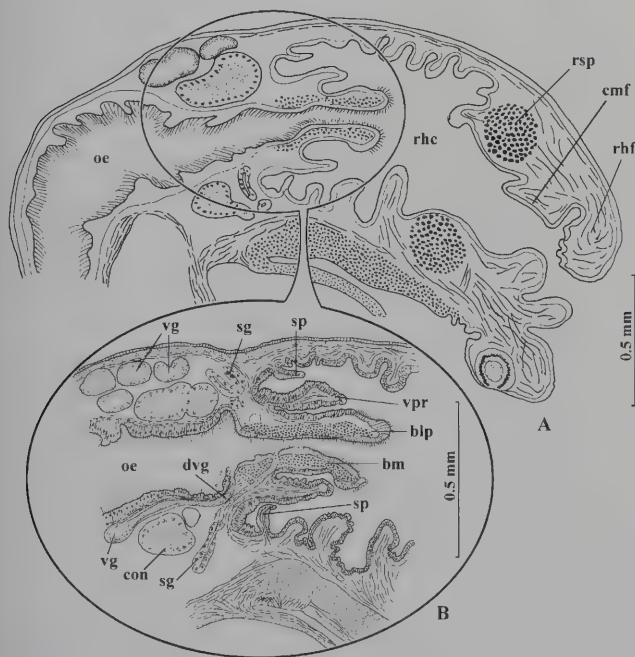
Figure 5. *Pseudodaphnella granocostata* (Reeve, 1846). **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged region of the posterior rhynchodaeum and buccal mass.

Glands

The unpaired salivary gland is large, tubular and highly convoluted. The walls of the gland are formed by a single type of large, oval cell with granulated cytoplasm, which bears long cilia. Anteriorly, the gland gradually passes into a narrow ciliated duct, which opens ventrally at the border of posterior third of the buccal mass.

The venom gland is long and convoluted, with a change in histology prior to its anteriorwards passage through the nerve ring into a narrow ciliated duct, which opens just posterior to the buccal mass. The diameter and histology of the venom duct are similar to that of the salivary duct. The muscular bulb is very small, of the same diameter as the venom gland and with the wall formed from a single, thin layer of longitudinal muscle fibres. Internally the bulb is lined with an low epithelium bearing very long cilia.

Radula

The radula is absent.

Thatcheria mirabilis Angas, 1877
(Figures 3 I, 6, 20F)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is large and located posteriorly within the rhynchostome. To the anterior there is a well-developed rhynchostomal funnel, rhynchostomal introvert absent. The epithelium lining the anterior half of the rhynchocoel consists of tall, columnar, ciliated cells but in the posterior half this is gradually replaced by low, smooth epithelium. The rhynchodaeum is thick-walled and folded.

In the posterior part of the rhynchocoel there is a thin but highly muscular septum with a relatively narrow orifice. While the outer side of the septum is lined with an epithelium of low, cubical cells, the inner side, as well as the rest of rhynchodaeum and proboscis walls is lined with tall, columnar, non-ciliated cells. In the retracted state the proboscis can appear very short, lying posterior to the septum, but it is capable of significant extension and in one dissected specimen was seen protruding through the septum orifice and occupied more than half the length of the rhynchocoel.

The mouth is broad. The buccal tube has neither sphincters nor any sac-like enlargement. The thin walls of the tube are lined with a low, smooth epithelium and highly folded due to the great contraction of the proboscis. The muscles of the proboscis walls are equally developed along their length. Defined proboscis retractor muscles absent.

Buccal mass and oesophagus

The buccal mass is large, broad, very thin-walled, and lies behind and outside of the proboscis. In the sectioned specimen, the buccal mass was distorted due to the great proboscis
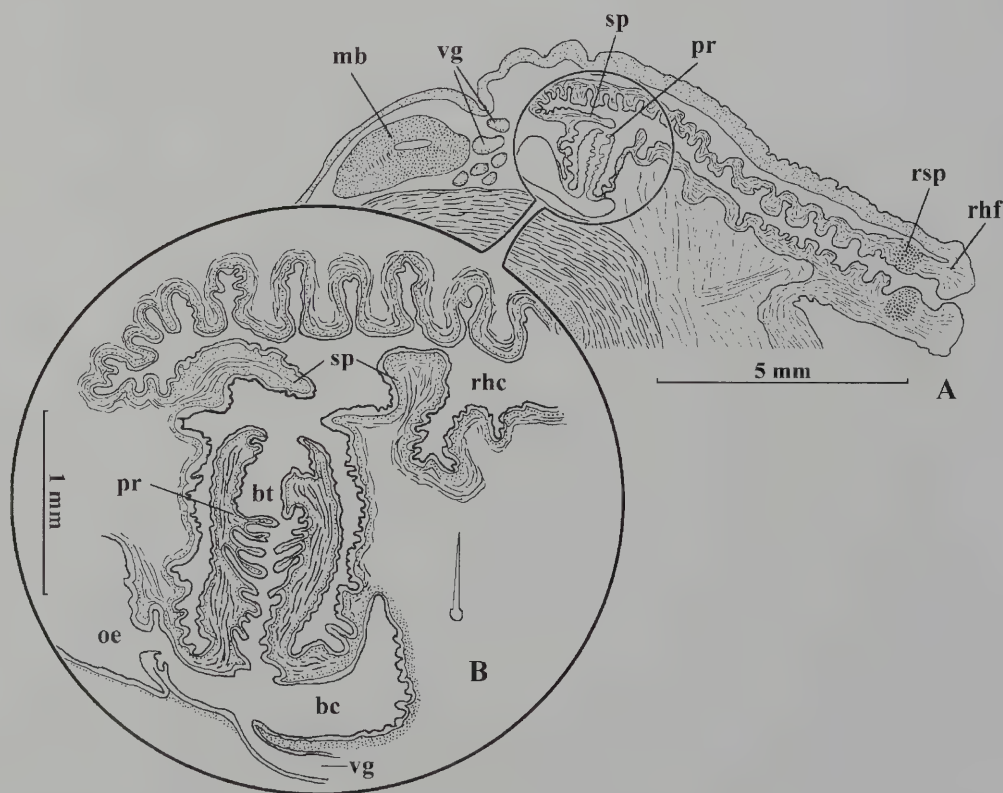
Figure 6. *Thatcheria mirabilis* Angas, 1877. **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged region of the proboscis and buccal mass (the single marginal tooth is drawn in the same scale to the right of the proboscis).

contraction. The buccal mass gradually passes into a wide oesophagus without an obvious border. It is lined with a medium-tall, ciliated epithelium.

Glands

The salivary glands are paired, tubular, long, highly convoluted and wide (about 100 μm in diameter). The epithelium, lining the glands comprises uniform, very low, non-ciliated cells. The glands gradually pass into the ducts, which are much narrower (about 30 μm in diameter) and lined with ciliated epithelium. The ducts open into the radular sac close to its entry into the buccal cavity.

The venom gland opens ventrally at the posterior border of the buccal cavity and does not change in histology after passing anteriorly through the nerve ring. The muscular bulb is large, long and oval, with the wall formed from a single, thick layer of circular muscle fibres. The lumen of the bulb is narrow and lined with a low, non-glandular epithelium.

Radula

The radular sac is small and short, and situated laterally to the buccal cavity, opening on the right side through a short but wide buccal sac. The radular teeth (Fig. 3I) are very small, about 280 μm long in a specimen with a shell length 88 mm, that is 0.3% of the shell length The teeth are enrolled, and awl shaped with a large adapical opening and an extended base.

Hemilienardia malleti (Récluz, 1852)
(Figures 3H, 7, 8, 20L)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is medium-sized and long. There is very large rhynchostomal introvert (Fig. 7) which, in the retracted position, occupies nearly the entire length of the rhynchocoel. There is no rhynchodeal septum.

The retracted proboscis is narrow, long, highly convoluted and probably exceeds the rhynchocoel in length (it is shown less coiled and shorter on the figure). The proboscis is attached near the middle of the ventral wall of the rhynchodaeum. The proboscis lumen is filled with oval cells with large nuclei. Defined proboscis retractor muscles absent. The mouth is very narrow. The buccal tube is thin-walled, with a small sac-like enlargement near the mouth, which is lined by tall columnar epithelium, forming a small pad. A single radular tooth was seen held at the proboscis tip with the base adhering to the pad. Prominent buccal tube sphincters are absent. The buccal tube is lined with very low, non-ciliated epithelium.

Buccal mass and oesophagus

The buccal mass is poorly defined, short and thin walled. It is lined with a columnar epithelium with very long cilia and passes gradually into the oesophagus.

Glands

The salivary glands are paired, tubular, long and highly convoluted and relatively rather thick (about 22 μm in diameter). The epithelium, lining the glands is of uniform, columnar, ciliated cells, completely occupying the lumen of the gland.
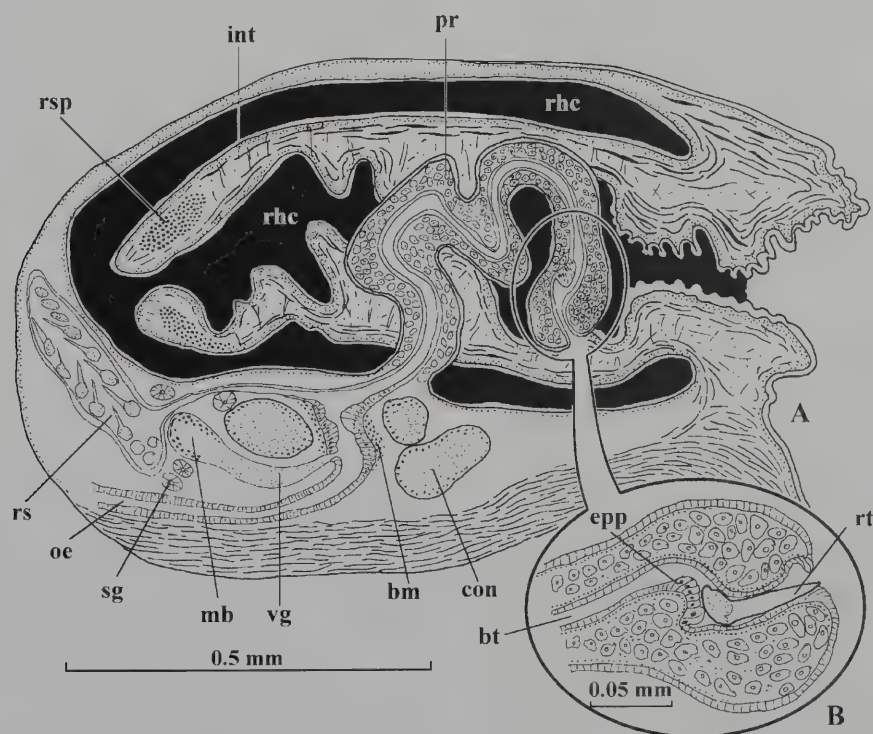
Figure 7. *Hemilienardia malleti*. A. Semidiagrammatic longitudinal section of the foregut (rhynchocoel - solid black). B. Enlarged proboscis tip with the marginal tooth.

The venom gland is very short (entire gland is shown on figure 7A - vg), of uniform histology, and slightly coiled with a diameter of about 45 µm. It opens into the buccal cavity in the area of the nerve ring, so that the gland itself does not pass through the ring. The muscular bulb is small and oval, with a diameter about the same as the gland, and the wall formed by a single, thin layer of muscle fibres.

Radula

The radular sac is relatively large and long and the radular caecum small. The buccal sac is long, narrow and curved. The radular teeth (Fig. 3H) are robust, relatively long (80 µm), broad, with a pointed tip and a large extended base.

Paramotana rufozonata (Angas, 1877)
(Figures 3J, 9, 20K)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is medium-sized and positioned posteriorly within the rhynchostome. Rhynchostomal introvert or funnel absent. The tall, columnar epithelium of the rhynchocoel is cuticularized, with very large oval cells. There is a septum in the posterior part of rhynchocoel. The epithelium, lining the outer surface of the septum is continuous with that of the rhynchodaeum, while on the inner surface it is replaced by similar but lower epithelium, which is continuous with that of the proboscis wall. Between the body wall and dorsal wall of the rhynchocoel there are numerous very large, irregularly oval cells with granulated cytoplasm and large oval nuclei (Fig. 9A – glc). These cells are probably glandular.

The proboscis is short and in the contracted state occupies less than half the length of the rhynchodaeum. Defined proboscis retractor muscles absent. The mouth opening is very narrow and surrounded by a circular fold of the proboscis wall. The buccal tube is narrow, thin-walled and widens at the proboscis base. It is lined with a low cuticularized epithelium. There are no sphincters.

Figure 8. *Hemilienardia malleti*. Extended rhynchodeal introvert, relaxed specimen, critical point dried. Scale bar = 100µm.

Buccal mass and oesophagus

The buccal mass is very small, with rather thin walls, forming large buccal lips which protrude into the buccal tube (Fig. 9B - blp). The buccal cavity is lined with medium-tall cells possessing long cilia. To the posterior and before passing into oesophagus the walls of the buccal cavity form a posteriorly-projecting circular fold. This is lined with tall, columnar, epithelial cells, which bear extremely long cilia, that form a valve (Fig. 9C). The epithelium of the oesophagus is low and ciliated.

Glands

The salivary gland is probably unpaired, tubular and coiled. The walls of the gland are formed from a single type of large, irregularly oval, ciliated cells. These have a granulated cytoplasm and oval nuclei situated at the base or in the middle of the cells. The venom gland is long, convoluted and thick, and does not change in histology after passing through the nerve ring. It opens into the buccal cavity immediately posterior to the buccal sac. The muscular bulb is very small, of the same diameter as the venom gland, with the wall formed of a single thin layer of circular muscle fibres. Internally the bulb is lined with a low, smooth epithelium.

Radula

The radula sac is medium-sized, situated to the left of the proboscis base. The buccal sac which is very narrow, long and curved, opens ventrally into the buccal cavity, just anterior to the venom gland. The radular teeth (Fig. 3J) are about 40 µm long, simple, enrolled, awl shaped with a pointed tip, a narrow channel near the tip and a large extended base.

Kermia barnardi (Brazier, 1876)
(Figures 10, 20I)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is medium-sized, and located posteriorly in the rhynchostome. Rhynchostomal introvert or
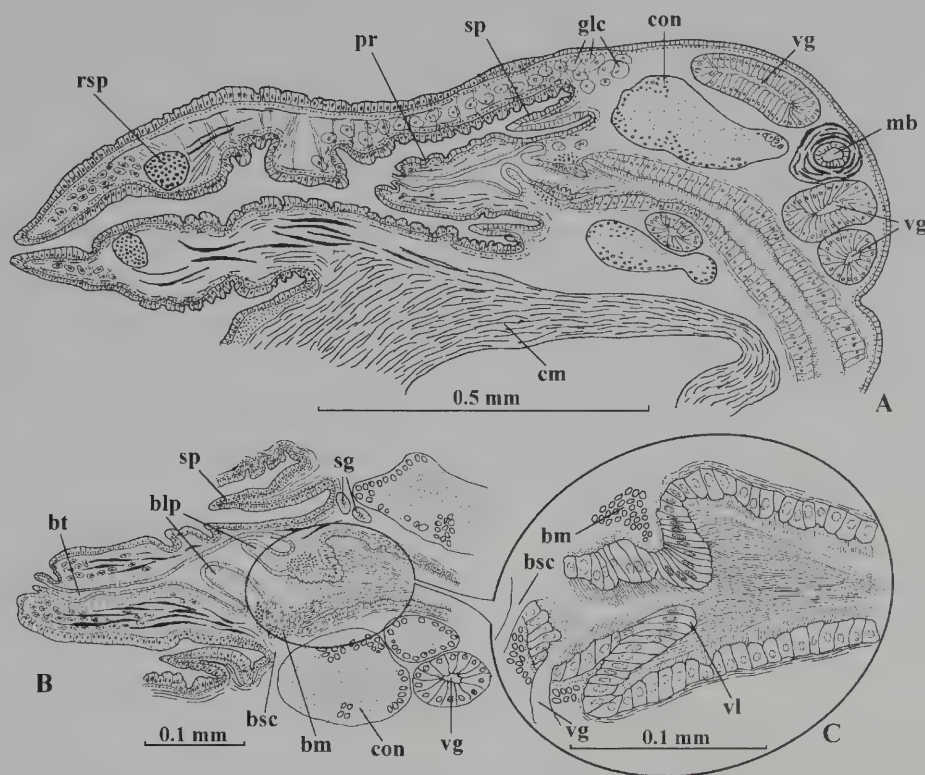
Figure 9. *Paramotana rufozonata* (Angas, 1877). A. Semidiagrammatic longitudinal section of the foregut. B. Enlarged proboscis and buccal mass. C. Enlarged buccal mass with the valve.
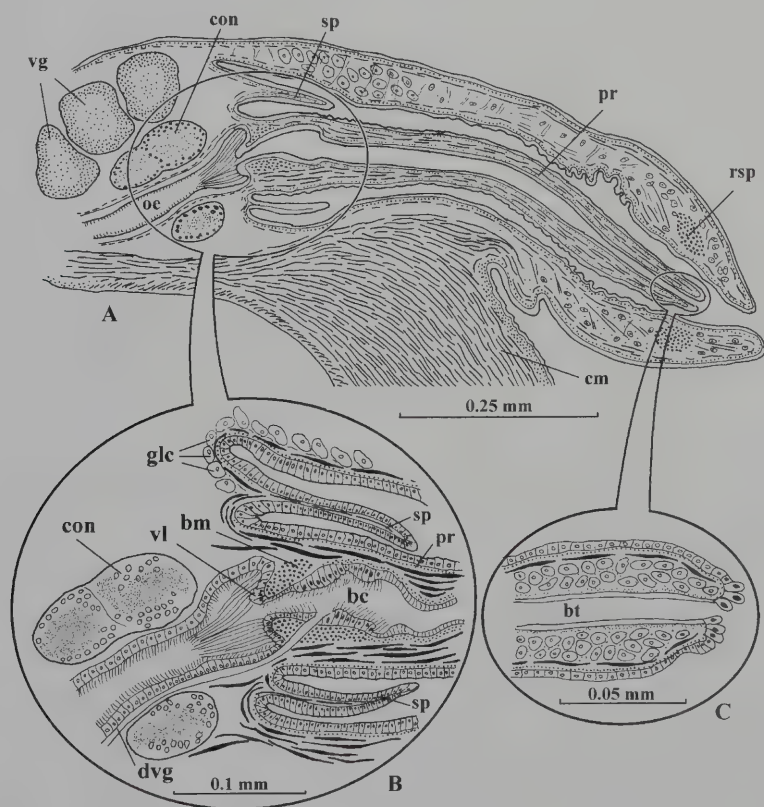
Figure 10. *Kermia barnardi* (Brazier, 1876). A. Semidiagrammatic longitudinal section of the foregut. B. Enlarged proboscis base and buccal mass (opening of the buccal sac not shown). C. Enlarged proboscis tip.

funnel absent. The rhynchocoel possesses a cuticularized, epithelium of tall, columnar cells with very large oval nuclei that occupy most of the cell length. In the posterior part of the rhynchocoel there is a small septum. The epithelium lining the septum and proboscis is continuous with that of the rhynchodaeum. Between the body wall and dorsal wall of rhynchocoel there are numerous very large, irregularly oval cells with a granulated cytoplasm and relatively small oval nuclei (Figure 10B - glc). These are probably glandular cells.

The proboscis is long and in the contracted state occupies the entire length of the rhynchodaeum. Proboscis retractor muscles run along the proboscis walls. The border between the muscles and the wall is unclear so that retractors give the appearance of a thick proboscis wall. The mouth opening is very narrow and surrounded by tall goblet-shaped cells, possibly sensory, with large oval nuclei and granulated protoplasm. The buccal tube is very narrow and thin-walled anteriorly but becomes wider and thicker walled posteriorly. There is no anterior sphincter.

Buccal mass and oesophagus

The buccal mass is very small with rather thin walls, and the buccal cavity is lined with tall cells with long cilia. Near the boundary with the oesophagus, the walls of the buccal cavity form a posteriorly-projecting fold. This is lined with tall, columnar, epithelial cells, with extremely long cilia, that form a valve. The epithelium of the oesophagus is composed of low, ciliated cells.

Glands

The salivary gland (s?) is small, tubular and coiled. The gland walls are formed from a single type of large, irregularly-oval cells with granulated cytoplasm and oval nuclei situated either at the base or in the middle of the cells. The lumen of the gland is very narrow.

The venom gland is long, convoluted and thick. There is a change in histology just prior to its passage through the nerve ring where it forms a much narrower, ciliated duct, which passes through the ring and opens into the buccal cavity immediately posterior to the buccal sac. The muscular bulb is small, of slightly larger diameter than the venom gland, with the wall formed from a single thin layer of circular muscle fibres. Internally the bulb is lined with a low, smooth epithelium.

Radula

The radular sac is medium-sized and situated latero-ventrally to the left of the proboscis base. The buccal sac is short, narrow and straight and opens into the buccal cavity ventrally.

No radular teeth were obtained.

Phymorhynchus wareni Sysoev & Kantor, 1995
(Figures 11, 20D)

The description is modified from Sysoev & Kantor (1995).

Rhynchodaeum and proboscis

The rhynchodeal cavity is long, with a large, posteriorly located, rhynchostomal sphincter, and with very large, rhynchostomal funnel. The walls of the funnel are highly folded and capable of great extension. There are well-developed transverse muscles connecting the rhynchodaeum with the body walls. A thick, muscular septum is located in the posterior part of rhynchocoel. The proboscis is short and occupies about one
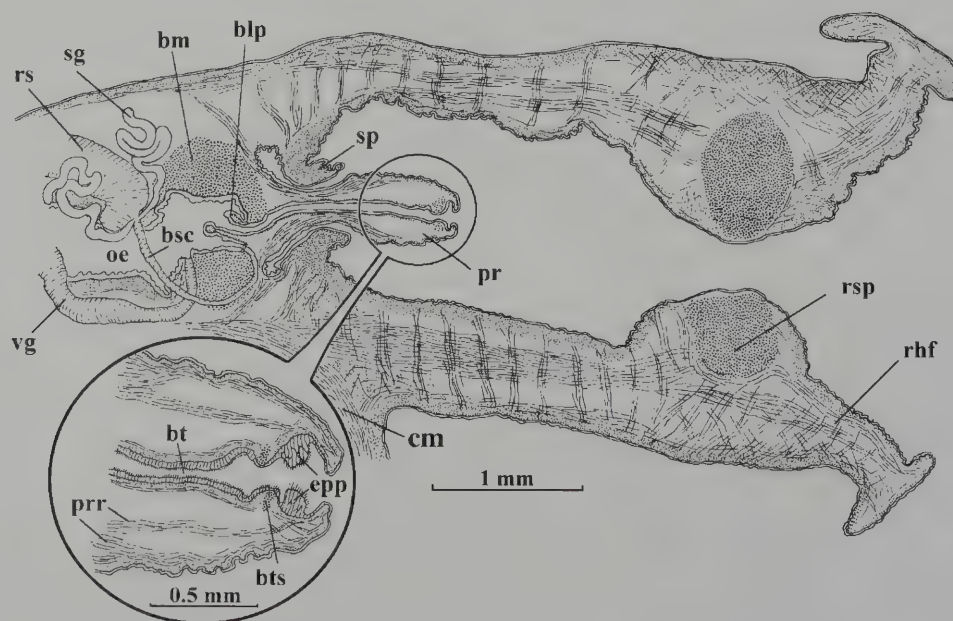
Figure 11. *Phymorhynchus wareni* Sysoev & Kantor, 1995 [after Sysoev & Kantor, 1995, slightly modified]. **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged proboscis tip.

third of the length of the rhynchodeal cavity. Its walls are covered by a low, cubic, cuticularized epithelium and internally formed by thin outer layer of circular muscle fibres and an inner layer of longitudinal fibres. The posterior quarter of the proboscis and the inner side of septum are lined with a tall, columnar epithelium. Massive proboscis retractor muscles are attached to the walls of the proboscis about half way along its length. In the posterior part of the proboscis, the retractor muscles occupy the whole inner lumen and after leaving it are then attached to the columellar muscle and roof of the body haemocoel. The proboscis retractor muscles give rise to smaller muscle bundles which run to the wall of the sac-like enlargement of the buccal tube.

The mouth opening is small and rounded and this leads to a sac-like enlargement of the buccal tube. The epithelial cells lining the enlargement are tall, bear long cilia, and form a pad. There is a small sphincter of the buccal tube at the base of the sac-like enlargement (Fig. 11B – bts). The buccal tube itself is rather thick-walled and lined with a ciliated epithelium. The wall of the buccal tube is formed of circular muscle fibres, underlain by a thin layer of longitudinal fibres. A single radular tooth was observed within the lumen of the buccal tube near the proboscis base.

Buccal mass and oesophagus

The large, bulb-shaped, buccal mass is situated at the base of the proboscis. There are small and thin-walled, buccal lips, inverted inside the buccal cavity. The oesophagus is wide, lined with tall cells with very long cilia and opens in the U-shaped stomach. The buccal cavity and the oesophagus are filled with unrecognisable food content.

Glands

Long, convoluted, tubular, salivary glands open by short, poorly differentiated ducts into the base of the long buccal sac.

The venom gland is long and convoluted and opens into the posterior part of the buccal cavity. There is no change in histology of the gland anterior to the nerve ring. The medium-sized, oval, muscular bulb is formed by a single, thick layer of circular muscle fibres.

Radula

The buccal sac opens in the anteriormost part of the buccal cavity. The sac is very long and narrow and runs along the right side of the buccal mass. The length of the sac is about eight times longer than a single radular tooth. The radular teeth have a rather short, slender shaft (mean tooth length = 0.380µm) with a prominent single barb at the distal tip and a complex base divided into a haft and three spurs (SYSOEV & KANTOR, 1995; Fig. 5 A-C).

Phymorhynchus moscalevi SYSOEV & KANTOR, 1995
(Figures 3 C-D, 12, 20E)

Description is based on SYSOEV & KANTOR (1995).

Rhynchodaeum and proboscis

The rhynchodeal cavity is long with a large rhynchostomal sphincter. Anteriorly, there is a large, highly muscular, rhynchostomal funnel with muscle fibres orientated in several directions. This structure resembles a muscular hydrostat (Kier, 1988) and the funnel is probably very mobile. Well-developed transverse muscles connect the rhynchodaeum with the body walls. At the posterior end of the rhynchocoel there
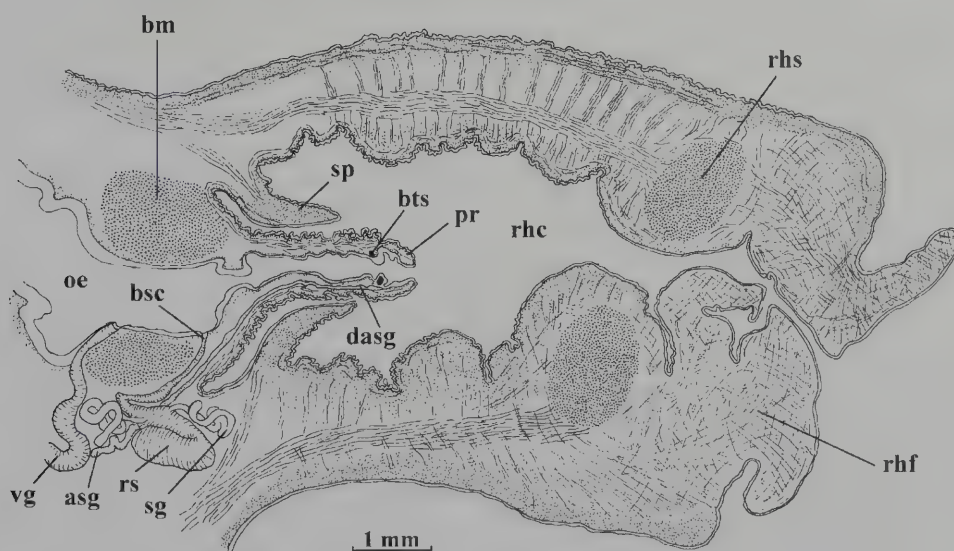
Figure 12. *Phymorhynchus moscalevi* Sysoev & Kantor, 1995 [after Sysoev & Kantor, 1995, slightly modified]. Semidiagrammatic longitudinal section of the foregut.

is a thick and muscular septum through which the proboscis can be protracted. The rhynchocoel and outer side of the septum are lined with an epithelium of tall columnar cells with large oval nuclei. This epithelium is replaced at the tip of the septum by one of low cubic cells, which is continuous with the proboscis epithelium.

The proboscis is very small and occupies about one-third the length of the rhynchodeal cavity. Well-developed retractor muscles run for almost the entire length of the proboscis and occupy nearly the whole inner lumen of the posterior part. To the posterior, they are attached to the columellar muscle and roof of the body haemocoel. A small and rounded mouth opening leads to a sac-like enlargement of the buccal tube. The epithelial cells, lining the enlargement are taller than that of the rest of the buccal tube and form a pad. There is a very small sphincter at the base of the sac-like enlargement. The thick wall of the buccal tube is formed of circular muscle fibres, with an underlying thin layer of longitudinal fibres. In the sectioned specimen the lumen of the tube was filled with particles of pyrites.

Buccal mass and oesophagus

The large bulb-shaped buccal mass is situated at the base of the proboscis. The buccal cavity is wide and only slightly narrower than the oesophagus.

Glands

A pair of long, highly convoluted, tubular, salivary glands open by short, poorly differentiated ducts into the base of the long buccal sac. Additionally, there is a small, tubular, convoluted, accessory salivary gland, situated below the buccal mass. The gland has histology typical of the Conoidea (Schultz, 1983; Taylor & Miller, 1990) and is formed of two epithelial layers divided by a thin layer of circular muscle fibres. It opens by a duct into the anterior, ventral part of the buccal tube.

The venom gland is long and convoluted and opens into the posterior part of the buccal cavity. The large, oval, muscular bulb is formed by a single thick layer of circular muscle fibres and has a wide lumen.

Radula

The buccal sac is long and narrow, forming a sharp bend
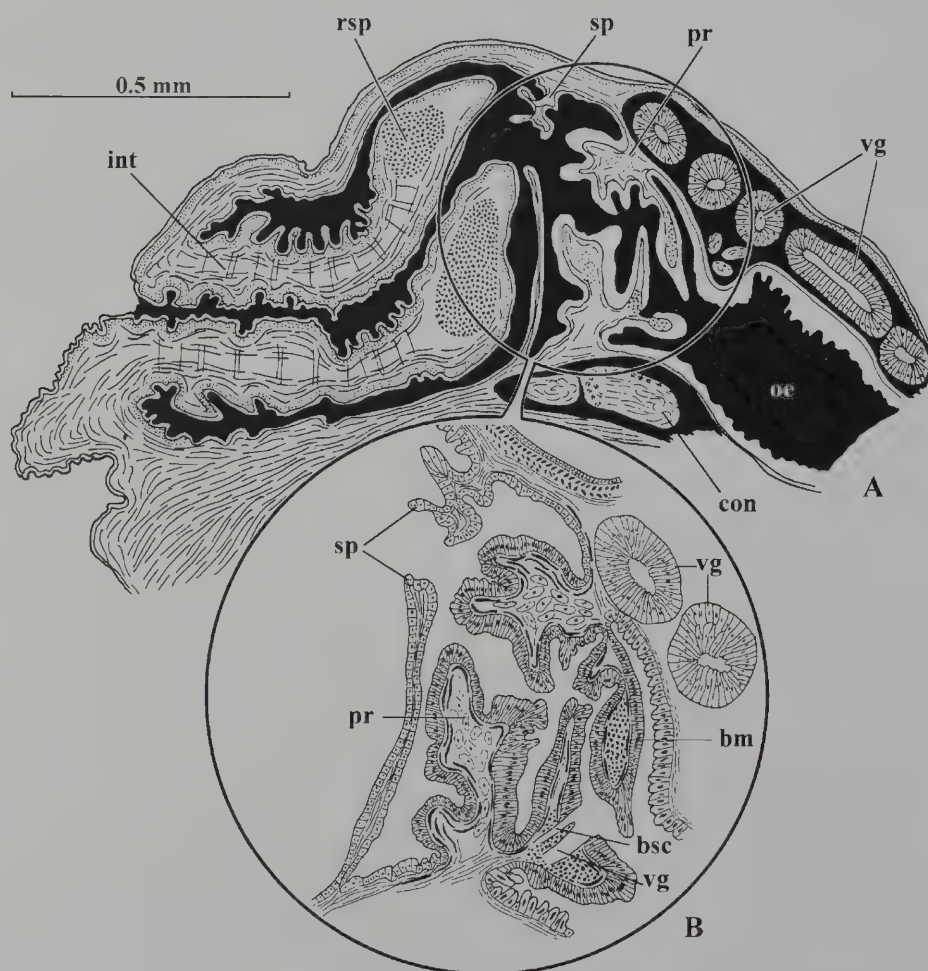
Figure 13. *Hemidaphne reeveana* (Deshayes, 1863) [after Taylor *et al.*, 1993, slightly modified]. **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged region of the proboscis and buccal mass.

after leaving the radular diverticulum and opens in the very anterior part of the buccal cavity. The radular sac is small, situated below the buccal mass. The radular teeth are long and slender (200 µm) with a narrow tip with a single barb and an extended complex base.

Hemidaphne reeveana (Deshayes, 1863)
(Figures 13, 20L)

Rhynchodaeum and proboscis

The rhynchostomal sphincter is large, situated at the tip of a large rhynchostomal introvert, which in the inverted position occupies most of the length of the rhynchocoel. Towards the posterior of the rhynchocoel there is a thin, poorly muscular, rhynchodeal septum with a relatively narrow orifice.

The proboscis, when retracted, is very short and lies posterior to the septum. Its walls are folded, which suggests the possibility of a great extension of proboscis length on protraction. Defined proboscis retractor muscles absent.

The mouth is broad and leads to a sac-like enlargement of the buccal tube at some distance from the mouth opening, which is lined by tall columnar epithelium, forming a pad. The buccal tube is thin-walled and folded, without sphincters and lined with columnar epithelium.

Buccal mass and oesophagus

The buccal mass is short and thin-walled. There are medium sized extensible buccal lips.

Glands

The salivary glands are paired, tubular, long and convoluted.

The venom gland does not change histology after passing anteriorly through the nerve ring and opens ventrally into the buccal cavity immediately posterior to the buccal sac. The wall of the muscular bulb is formed of single thin layer of circular muscle fibres.

Radula

Radula present but no details as only a single specimen was available for study.

Teretiopsis abyssalis Kantor & Sysoev, 1989
(Figure 14, 20H)

Description modified from Kantor & Sysoev, 1989.

Rhynchodaeum and proboscis

The rhynchostomal sphincter is large and located posteriorly within the rhynchostome. There is medium-sized rhynchostomal funnel. The epithelium of the rhynchocoel is tall, columnar and brown. There is no septum. The rhynchodaeum is connected to the body wall by numerous transverse muscles (Figure 14C - tm).

The proboscis is vestigial and represented by only a low circular fold at the base of the small extensible buccal lips. The proboscis is lined with a low epithelium. The buccal tube is absent due to the reduction of the proboscis.

Buccal mass and oesophagus

The buccal mass is medium-sized with rather thick walls. The buccal cavity is very narrow and lined with a low, smooth epithelium. The oesophagus is wide and lined with tall epithelium.
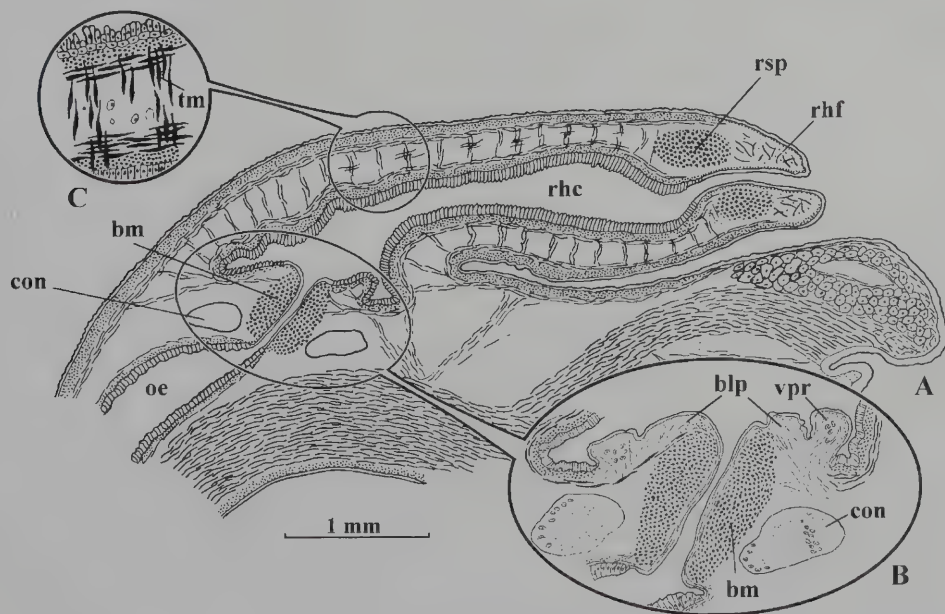
Figure 14. *Teretiopsis abyssalis* Kantor & Sysoev, 1989 [after Kantor & Sysoev, 1989, modified]. **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged region of the proboscis and buccal mass. **C.** enlarged part of the rhynchodaeum wall.

Glands
There are no foregut glands present.

Radula
Absent.

Teretiopsis levicarinatus KANTOR & SYSOEV, 1989
(Figures 15, 20G)

Description modified from KANTOR & SYSOEV, 1989.

Rhynchodaeum and proboscis
The rhynchostomal sphincter is large and located to the posterior of the rhynchostome. There is medium-sized rhynchostomal funnel. The epithelium of the rhynchocoel is tall, columnar and dark-brown. There is no septum. The rhynchodaeum is connected with the body wall by numerous transverse muscles.

The proboscis is vestigial and represented by only a low circular fold at the base of the small extensible buccal lips. The proboscis and anterior surface of the buccal lips are lined with a rather tall epithelium of goblet-shaped cells. A buccal tube is absent due to the reduction of the proboscis.

Buccal mass and oesophagus
The buccal mass is very small with thin walls. The buccal cavity is very narrow and lined with very low epithelium. The epithelium of the oesophagus is dark brown and tall, somewhat similar to that of the rhynchocoel.

Glands
There are no foregut glands.

Radula
Absent.

Teretia teres (Forbes, 1844)
(Figure 20N)

The sectioned specimen was rather poorly fixed but some details of the anatomy are given below and characters included in the matrix (Table 1).

Rhynchodaeum and proboscis
There is a long rhynchodeal introvert which in the retracted state, occupies about 2/3 of the rhynchocoel. The rhynchostomal sphincter is large and situated at the introvert tip. A prominent septum with a narrow central aperture is situated towards the posterior of the rhynchocoel. The proboscis is short and occupies about 1/4 of the rhynchocoel. A distal buccal tube sphincter is present.

Buccal mass and oesophagus
The buccal masss is short and thin walled. No buccal lips were observed.

Glands
The salivary glands are paired and tubular. The venom gland is prominent with no change in histology anterior to the nerve
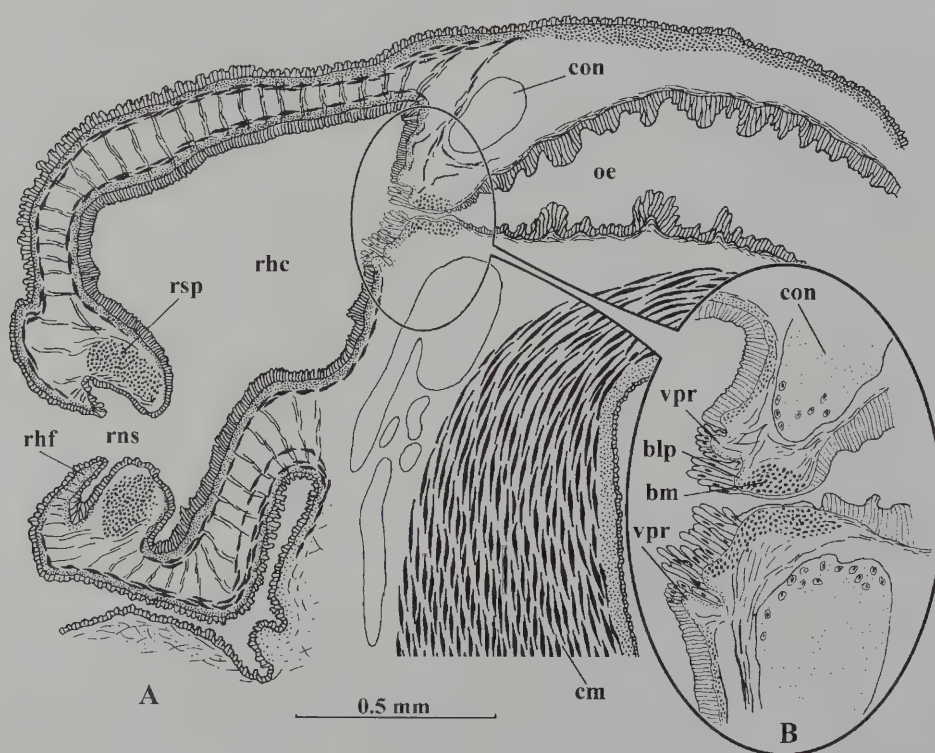
Figure 15. *Teretiopsis levicarinatus* Kantor & Sysoev, 1989 [after Kantor & Sysoev, 1989, modified]. **A.** Semidiagrammatic longitudinal section of the foregut. **B.** Enlarged region of the proboscis and buccal mass.

ring. The muscular bulb consists of a single layer of circular muscle with the lumen lined with a low epithelium.

Radula

BOUCHET & WARÉN (1980 p. 82) state that the radula is absent in this species but the radular sac and teeth are clearly seen in the thin sections. The individual radular teeth are short, enrolled and with large bases.

CHARACTER STATES AND PHYLOGENETIC ANALYSIS

In addition to the species described and illustrated above, a few other species of Raphitominae for which sufficient anatomical details have been published have also been included in the analysis. These species are *Pontiothauma mirabile* Smith, 1895 (PACE 1903, and radular details Fig. 3E); *Philbertia purpurea* (MONTAGU, 1803), *P. linearis* (MONTAGU, 1803) (details in SHERIDAN *et al.*, 1973); *Caenodagreutes aethus* Smith, 1967 (SMITH, 1967a). The characters and their states are briefly reviewed below and their distribution amongst the taxa shown in Table 2. A particular difficulty with raphitomines is the reduction or absence loss of foregut organs and this causes problems in the coding of inapplicable character states. Following STRONG & LIPSCOMB (1999) we have coded inapplicables as "?" (reductive coding). Moreover, a major limitation of the analysis is the fact that only a very small proportion of the living genera and species have been studied.

1. Rhynchostomal introvert (Fig. 1A, 7A - int): 0 = absent; 1 = present

The introvert is a mobile elongation of the rhynchostomal lips which when retracted lies within the rhynchodeal cavity but extends as a tube when protracted. Other than some species of raphitomines an introvert has been otherwise recorded only in the Terebridae.

2. Rhynchostomal funnel: 0 = absent; 1 = present

The funnel is formed by a muscular extensions of the anterior part of the rhynchostome (Fig. 11A, 12 - rhf). The rostrum of some *Conus* species is a similar structure.

3. Rhynchodeal septum: 0 = absent (Fig. 7, 14, 15 – sp); 1 = mid-rhynchodaeum (Fig. 1, 13 – sp); 2. posterior rhynchodaeum (Fig. 5B, 9A, 10A – sp).

Many raphitomines possess a thin muscular septum that divides the rhynchocoel. This is situated either towards the middle of the cavity or more usually at the posterior. Similar septa are found in some Terebridae and a few *Conus* species (Fig. 16, 17A - sp).

4. Proboscis: 0 = long; 1 = short; 2 = vestigial; 3 = absent

Most conoideans have a long proboscis (occupying more than half the length of the rhynchodaeum when retracted) but in raphitomines and some Terebridae it may be reduced or absent.

Table 2. Matrix of characters and character states for raphitomine species and outgroups.

Character ***Taxon***	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Gymnobela emertoni	0	0	2	2	?	?	?	?	0	0	2	1	1	1	?	?	0	1	0
Gymnobela pyrrhogramma	1	1	1	1	0	0	1	1	0	0	2	1	1	0	0	2	0	1	0
Gymnobela sp.	0	0	0	0	0	1	0	1	0	1	?	1	0	0	0	1	0	1	?
Teretia teres	1	0	1	1	0	?	1	1	?	0	2	1	1	0	0	2	0	1	0
Teretiopsis abyssalis	0	1	0	2	?	?	?	?	0	1	?	2	1	1	?	?	0	1	0
Teretiopsis levicarinatus	0	1	0	2	?	?	?	?	0	1	?	2	1	1	?	?	0	1	0
Kermia barnardi	0	0	2	0	0	1	1	1	1	0	2	1	1	0	1	2	1	1	0
Hemidaphne reeveana	1	0	1	1	0	1	1	1	0	0	2	1	1	0	0	2	0	1	0
Pseudodaphnella granicostata	0	1	2	2	?	?	?	?	0	1	?	1	1	0	1	2	0	1	0
Paramontana rufozonata	0	0	2	1	0	1	1	1	0	0	2	1	1	0	0	2	1	1	1
Thatcheria mirabilis	0	1	2	1	0	1	1	1	1	0	2	1	1	0	0	2	0	1	0
Phymorhynchus moscalevi	0	1	2	1	0	0	1	1	1	0	2	1	0	0	0	2	0	1	?
Hemilienardia malleti	1	0	0	0	0	1	1	1	1	0	2	1	1	0	0	2	0	1	0
Pontiothauma mirabile	0	1	2	1	0	?	1	1	1	0	2	1	1	0	0	2	0	1	?
Philbertia purpurea	1	0	1	0	0	1	1	1	0	0	2	1	1	0	0	2	0	1	*0*
Philbertia linearis	1	0	0	3	?	?	?	?	0	1	?	2	1	1	?	?	0	1	0
Caenodagreutes aethus	0	0	?	2	?	?	?	?	0	1	?	2	1	1	?	?	0	1	0
Conus boholensis	0	1	1	0	0	1	0	1	1	0	3	0	1	0	0	0	0	0	1
Hastula bacillus	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1
Mangelia nebula	0	0	0	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	1
Ophiodermella inermis	0	0	0	0	0	0	1	1	0	0	3	0	0	0	0	1	0	0	1
Terebra subulata	1	0	1	0	0	0	1	1	1	0	3	0	0	0	0	3	0	0	1
Oenopota levidensis	0	0	0	1	0	1	1	1	0	0	2	1	1	0	0	1	0	0	1

In some species, the low cylinder of muscular tissue surrounding the entrance to the buccal cavity represents a vestigial proboscis (Fig. 2B, 14B, 15B - vpr). Where the proboscis is vestigial or absent we have coded the following characters (5 - 8) as inapplicable "?".

5. Buccal tube introvert: 0 = absent; 1 = present.

This is a muscular flap-like structure located at the distal end of the buccal tube in the outgroup *Mangelia nebula* and other mangeliines (TAYLOR *et al.* 1993, fig. 9)

6. Anterior buccal tube sphincter (Fig. 1B, 11B, 12 - bts): 0= present 1 = absent

Most conoideans have a sphincter located in a distal position within the buccal tube.

7. Mid-buccal tube sphincter: 0 = present; 1 = absent

A sphincter in a middle position of the buccal tube found in *Conus* species (Fig. 1, 17B – bts).

8. Posterior (or basal) buccal tube sphincter (TAYLOR *et al.*,1993, fig. 9 - ps): 0 = present: 1 = absent.

A basal buccal tube sphincter is present in the outgroup *Mangelia nebula* (SHERIDAN *et al.*, 1973) and other Mangeliinae.

9. Buccal lips (Fig. 1B, 2B, 5B, etc. - blp): 0 = present; 1 = absent.

These are muscular extensions of the anterior walls of the buccal mass which project as a tube into the buccal tube. In some species they can be inverted into the buccal cavity. Buccal lips occur sporadically throughout the Conoidea (TAYLOR *et al.*1993; KANTOR *et al.* 1997).

10. Radula: 0 = present; 1 = absent.

11. Radula – type of marginal teeth: 0 = semi-enrolled; 1 =semi-enrolled with large base; 2 = enrolled with extended base; 3 = enrolled with narrow base.

The marginal teeth of raphitomines and the outgroups used in this analysis can be divided into 4 types. In *Hastula bacillus* the teeth are semi-enrolled with a narrow base (TAYLOR *et al.*, 1993, fig. 22b); *Mangelia nebula* has semi-enrolled teeth with a large base (TAYLOR *et al.*, 1993; fig. 23 e & f). Most raphitomines have enrolled teeth with an extended base whilst *Conus* and *Terebra subulata* have enrolled teeth with a narrow base (Fig. 3G).

12. Salivary glands: 0 = acinous; 1 = tubular; 2 = absent.

In most conoideans the salivary glands are acinous in histology but in Mangeliinae, Raphitominae and a few species of Crassispirinae the glands are tubular.

13. Accessory salivary gland: 0 = present; 1 = absent.

A well-known apomorphy of the Neogastropoda, (PONDER, 1974; BALL, TAYLOR & ANDREWS, 1997), accessory glands are patchily distributed amongst the Conoidea.

14. Venom gland and muscular bulb: 0 = present; 1 =absent.

15. Histology of anterior venom gland: 0 = uniform histology; 1 = changes to a duct after passage through nerve ring.

In most conoideans the venom gland has a uniform histology along its entire length. In a few species of Turridae and some raphitomine species the gland changes to a ciliated duct after passing anteriorly through the circumoesophageal nerve ring.

16. Muscular bulb layers: 0 = 2 equal layers; 1 = very thin outer layer; 2 = single layer; 3 = outer layer thicker than inner.

In most conoideans the terminal muscular bulb of the venom apparatus consists of two muscle layers of more or less equal thickness divided by a thin connective tissue layer. In Mangeliinae the bulb comprises two layers but the outermost is very thin. Most raphitomines have a bulb composed of a single layer only. In *Terebra subulata* (an outgroup) the outer layer is much thicker than the inner.

17. Oesophageal valve (Fig. 9C, 10B - vl): 0 = absent; 1 = present.

The oesophageal valve was present only in two species of raphitomines. It occupies a similar position and has some structural similarity to the valve of Leiblein of Rachiglossa (GRAHAM, 1941) although the homology is uncertain.

18. Operculum: 0 = present; 1 = absent

19. Protoconch ornament: 0 = cancellate ornament; 1 = non-cancellate ornament

Outgroups

Except for *Conus boholensis* (described below) and *Conus ventricosus* (Fig. 16, modified from TAYLOR *et al* 1993, fig. 7) details of the species used as outgroups in the analysis may be found in TAYLOR *et al.* (1993) and the other references cited. Two species of Terebridae were included in the analysis because of the presence of the rhynchodeal introvert and septum in some species of the family.
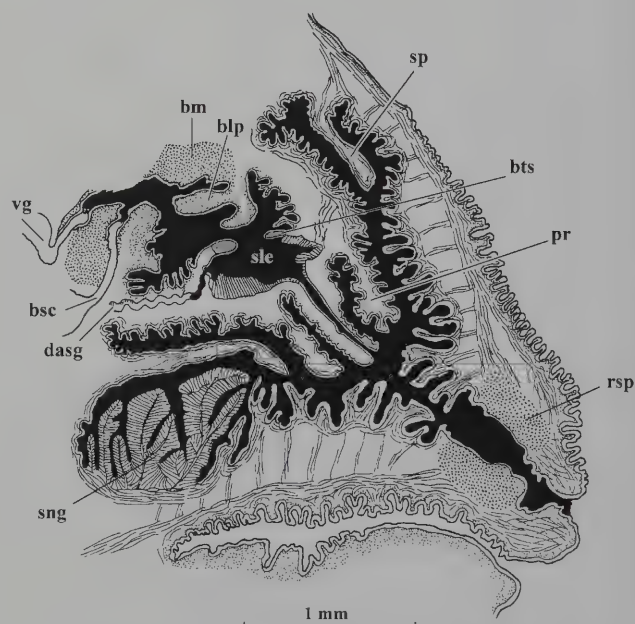
Figure 16. *Conus ventricosus.* Semidiagrammatic longitudinal section of the anterior foregut (after Taylor *et al.* 1993 modified).

Coninae:
Conus boholensis Petuch, 1979 (Fig. 17)
Conus ventricosus Gmelin, 1791 (Taylor *et al.*, 1993 and Fig. 16)
Clathurellinae:
Ophiodermella inermis (Hinds, 1843)
Mangeliinae:
Mangelia nebula (Montagu, 1803) (Sheridan *et al.*, 1973)
Oenopotinae:
Oenopota levidensis (Dall, 1919) (Shimek, 1975)
Terebridae:
Hastula bacillus (Deshayes, 1859) (Taylor & Miller, 1990)
Terebra subulata Linnaeus, 1767 (Taylor 1990)

Description of ***Conus boholensis*** Petuch, 1979
(Figures 3G, 17)

This species has a tall spire, a character usually considered primitive within the Coninae and also there is a rhynchodeal septum a hitherto unrecorded structure in *Conus*.

Rhynchodaeum and proboscis

The rhynchodeal cavity is long, with an anterior rhynchostomal sphincter of medium-size. A pronounced rhynchostomal funnel is absent, although the rhynchostomal lips are large. Transverse muscles, connecting the rhynchodaeum with the body walls are absent. In the medial part of the rhynchocoel there is a thin septum with a rather narrow orifice. The anterior part of the rhynchocoel and the outer side of septum are lined with a very tall glandular epithelium. This epithelium forms high folds, that nearly completely fill the cavity. The epithelial cells are ciliated with small oval nuclei. At the tip of the septum there is a sharp change to the low and non-glandular epithelium that lines the posterior part of the rhynchocoel.

The proboscis is medium-sized, and in the contracted stage occupies about half of the rhynchocoel. Its walls are highly folded so that when protracted the proboscis is long. The proboscis is lined with a cuticularized, cubical epithelium. The mouth opening is small and rounded and leads to a sac-like enlargement of the buccal tube. The epithelial cells, lining the enlargement are taller than that of the rest of the buccal tube. There is no anterior buccal tube sphincter. At the proboscis tip a single radular tooth was observed protruding through the mouth opening. This tooth is probably held in place by contraction of muscular walls of the anterior proboscis as well as by the epithelium of the of the sac-like enlargement. There is a small intermediate sphincter of the buccal tube.

Buccal mass and oesophagus

The buccal mass is short, with rather thick walls and lies posterior to the proboscis. Small buccal lips are present. The oesophagus is narrow, lined with ciliated epithelium and forms a very long curve between the buccal mass and the nerve ring.

Glands

The salivary glands are fused, very large, acinous and lie to the left of the rhynchodaeum. The salivary ducts are paired, long and highly convoluted. The venom gland does not change in histology after passing anteriorly through the nerve ring and
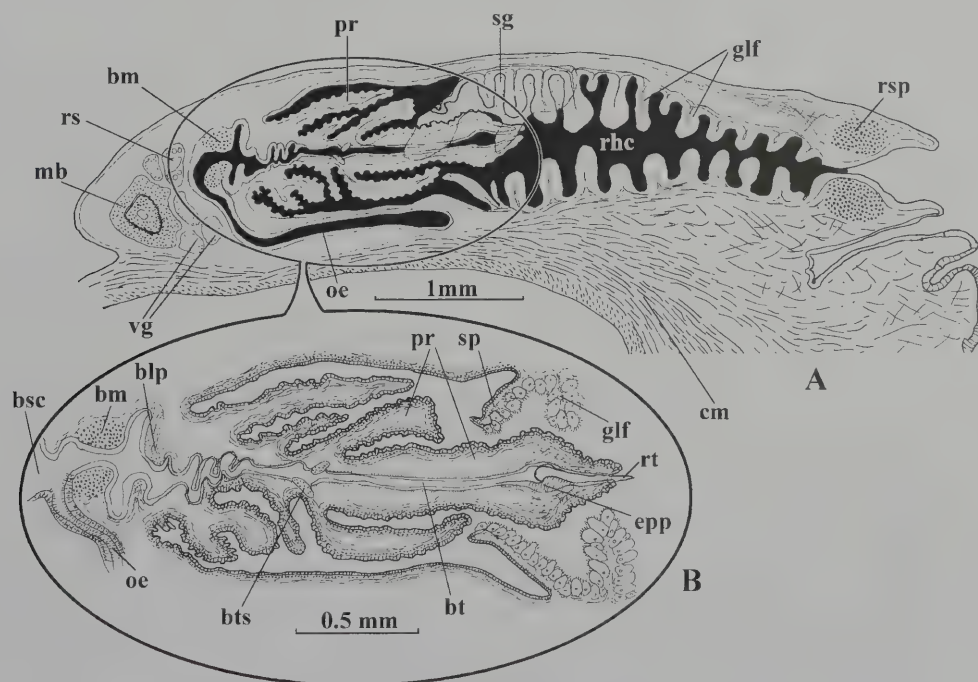
Figure 17. *Conus boholensis* Petuch, 1979. **A.** longitudinal section of foregut. Snout gland situated at the left side of the rhynchodaeum is seen as if the rhynchodaeum is transparent. **B.** Enlargement of posterior rhynchodaeum and buccal mass.

opens ventrally posterior to the buccal mass. The muscular bulb is very large, with its wall formed by two subequal layers of longitudinal muscle fibres, separated by a thick layer of connective tissue.

Radula

The radular sac is broad and long, and situated dorsally to the oesophagus (only the radular caecum is shown on Fig. 17A). It opens into the buccal cavity on the left side. The radular caecum is medium sized. The buccal sac (i.e. the portion of the radular sac between the entrance of the salivary ducts and the buccal cavity) is very long, narrow, and runs downward along the right side of the buccal mass, and then gradually turns towards its narrow opening ventrally into the buccal cavity just anterior to the sphincter of the buccal mass. The radular teeth (Fig. 3G) are long, at least 630 µm, slightly curved and hollow with two distal barbs.

Gymnobela emertoni
Gymnobela pyrrogramma
Teretia teres
Hemidaphne reeveana
Philbertia purpurea
Hemilienardia malleti
Gymnobela sp.
Teretiopsis abyssalis
Teretiopsis laevicarinatus
Philbertia linearis
Caenodagreutes aethus
Kermia barnardi
Pseudodaphnella grano.
Paramontana rufozonata
Thatcheria mirabilis
Phymorhynchus moscalevi
Pontiothauma mirabile
Conus boholensis
Hastula bacillus
Terebra subulata
Ophiodermella inermis
Mangelia nebula
Oenopota levidensis
OUTGROUPS
a

Figure 18. Strict consensus (a ↑) and 50% majority rule (b →) trees derived from 64 equally parsimonious trees with a length 52 steps. Consistency Index = 0.54, Rescaled C.I. = 0.74 and Retention Index = 0.4. Plain numbers indicate percentage occurrence of each clade in the set of equally parsimonious trees. Shaded numbers identify particular clades for reference.

Conus ventricosus Gmelin, 1791
(Figure 16)

The foregut anatomy of *Conus ventriciosus* is generally similar to *C. boholensis,* but the rhynchodeal septum is located in the posterior part of the rhynchodeal cavity. This structure was previously interpreted as a fold of the proboscis wall (TAYLOR *et al.*, 1993, fig. 7).

Parsimony analysis

The characters listed above and their states in the 17 species of Raphitominae and six outgroup species are given in Table 2. The analysis was performed using PAUP version 3.1.1. Multistate characters were treated as unordered. The heuristic search option was used with tree bisection-reconnection, branch swapping in effect and with ten replicates of a random addition sequence of taxa. The analysis produced 64 equally parsimonious trees of length 52 steps. The resulting strict consensus and 50% majority rule trees are shown in Fig.18.

The monophyly of the Raphitominae is supported at Clade 1 by the presence of radula teeth with large bases and the lack of an operculum. Clade 2 which includes all the Raphitomines examined, except *Gymnobela* sp., is supported by the three character states namely, the presence of diagonally cancellate

Gymnobela emertoni
Teretiopsis abyssalis
Teretiopsis laevicarinatus
Philbertia linearis
Caenodagreutes aethus
Pseudodaphnella grano.
Kermia barnardi
Paramontana rufozonata
Thatcheria mirabilis
Phymorhynchus moscalevi
Pontiothauma mirabile
Gymnobela pyrrogramma
Teretia teres
Hemidaphne reeveana
Philbertia purpurea
Hemilienardia malleti
Gymnobela sp.
Conus boholensis
Hastula bacillus
Terebra subulata
Ophiodermella inermis
Mangelia nebula
Oenopota levidensis
OUTGROUPS
1 100
2 75
3 100 100 100
4 75
5 100 100
6 75
7 75
8 100 88
b

Figure 19. Diagram summarising some of the major types of the foregut morphology, found among the Raphitominae. Not to scale. A, *Kermia barnardi*. B, *Paramotana rufozonata*. C, *Phymorhynchus* spp. (*P. moscalevi* with accessory salivary gland). D, *Thatcheria mirabilis*. E, *Gymnobella pyrhogramma* (with rhynchostomal sphincter shifted posterior from the tip of the introvert and with buccal tube sphincter), *Hemidaphne reeveana* (with rhynchostomal sphincter at the tip of the introvert and without buccal tube sphincter). F, *Hemilienardia malleti* and *Philbertia purpurea*. G, *Pseudodaphnella granicostata*. H, *Gymnobella emertoni*. I, *Teretiopsis* spp., *Philbertia linearis*, *Abyssobela atoxica* (without vestigial proboscis).

sculpture of the protoconch whorls (absent in *Paramontana*), the single muscle layer of the muscular bulb and the rhynchodeal septum (absent in several species). Clade 3 is supported in all trees by the possession of a rhynchodeal introvert, but this state is also found in *Philbertia linearis* (Clade 8). In Clade 4 the rhynchodeal septum is situated in a posterior position and lost in some of the terminal members of the clade. Clade 6 is supported by the anterior change in the histology of the venom gland in some species. Species in Clades 7 and 8 have lost major foregut organs (the radula, salivary glands and venom apparatus) and it is likely that this is not a natural grouping.

DISCUSSION

Our main objectives in this study were to describe details of foregut anatomy in a group of poorly known conoideans and normally it would be desirable to discuss the foregut evolution and traits of the Raphitominae within a framework of the phylogenetic analysis. However, although the monophyly of the Raphitominae is largely supported, the results from this (Fig. 18) are for several reasons, unsatisfactory. Firstly, only a small subset of the total diversity of raphitomines has been sampled and amongst these considerable disparity was found between taxa. Secondly, a feature of raphitomines is the reduction or loss of major foregut structures with the resulting loss of phylogenetic information derived from those structures. Thirdly, study of other organ systems or, more importantly, molecular analyses are necessary to corroborate or refute the results presented here.

Summary of anatomical variation in Raphitominae

Considerable variation was found in the configuration of the foregut amongst the species we studied. Nearly every species possesses a different type of foregut (Fig. 19), which differ in the presence, position and morphology of the main structures, such as proboscis, buccal mass, septum, glands and sphincters of the buccal tube.

There are very few characters, that are "characteristic" for the subfamily. The first of these is the morphology of the muscular bulb of the venom gland. In nearly all species in which the bulb is present its wall consists of a single layer of muscle fibres (usually of circular and rarely as in *Pseudodaphnella granicostata*, of longitudinally orientated fibres). The exception is *Gymnobela* sp., in which the wall of the muscular bulb is formed of two unequal layers of longitudinal fibres, separated by thin connective tissue layer. This morphology of the bulb is similar to that of species of Mangeliinae we have studied (TAYLOR *et al.*, 1993).

The other character (although not present in all species) is the septum, a more or less thin muscular, circular fold, pierced by an orifice, which divides the rhynchodaeum into two parts. Beside the raphitomines, the septum is found only in the subfamily Coninae and family Terebridae. The septum in raphitomines is usually positioned in the very posterior part of the rhynchocoel where it often resembles a circular fold at the proboscis base. In other species it is situated close to the middle of the rhynchocoel as in *Gymnobela pyrrhogramma*, *Hemidaphne reeveana*, *Teretia teres* and *Thatcheria mirabilis*. In this case the proboscis, in the retracted position, lies behind the septum, but can be protruded through the orifice when protracted (this was observed at least in *T. mirabilis*). In these species, it is likely that complete closure of the septal orifice is possible. In other species the septum may be greatly reduced and represented by very low fold of the rhynchodeal wall (*P. granicostata*, *Philbertia purpurea* (SHERIDAN *et al.* 1973), or be completely absent. The presence, position and degree of development of septum do not correlate with any other characters of the foregut. At present the function of the septum is obscure. In most species there is a change in the epithelial lining at the tip of the septum; the epithelium of the outer side of the septum is continuous with that of the rhynchocoel, while that on the inner side is confluent with the epithelium of proboscis wall. Thus the division of the rhynchocoel into two portions is probably of some functional significance. It is uncertain whether the septum is a plesiomorphic character for the all Conoidea and lost in the majority of taxa or if it has been evolved independently in the Terebridae and Conidae.

Raphitominae are the sole subfamily of Conidae, in which some species possess a rhynchostomal introvert. This structure is present in *Gymnobela pyrrhogramma*, *Hemidaphne reeveana, Hemilienardia malleti*, *Teretia teres* (our data), along with *Philbertia purpurea* and *P. leufroyii boothi* (SMITH, 1967a; SHERIDAN *et al.*, 1973). Apart from these occurrences in the Raphitominae, an introvert is found in most species of Terebridae (TAYLOR, 1990; SIMONE, 1999). Species of raphitomines, in which the introvert is absent are usually characterised by a greatly elongated head. Also in species without an introvert, the rhynchostome is frequently characterised by the formation of the so called "rhynchostomal funnel", which in effect comprises rather enlarged and usually highly muscular lips, which are probably capable of active movements. Although no direct observations have been made on the feeding of species with such a funnel, it is highly likely that it is employed in prey capture.

In the majority of species, whether or not they possess an introvert or funnel, there are transverse muscles, that connect the rhynchodaeum with the body walls. KANTOR & SYSOEV (1989) suggested, that during the contraction of the transverse muscles, the inner volume of the rhynchocoel increases and thus a negative pressure arises in the rhynchocoel. This possibly facilitates the suction of the prey through the rhynchostome.

The salivary glands of raphitomines are tubular and either paired or single. Usually the lumen of the gland is very narrow and their walls are formed by tall, ciliated epithelium. An exception is *Thatcheria mirabilis* in which the glands have a very wide lumen and the epithelium is low, cubic and non-ciliated.

In some species, namely *Kermia barnardi* and *Pseudodaphnella granicostata,* that portion of the venom gland situated in front of the circum-oral nerve ring narrows to form a duct. Such a duct is often found in species of the family Turridae (*sensu* TAYLOR *et al.*, 1993; KANTOR *et al.*, 1997). This transformation
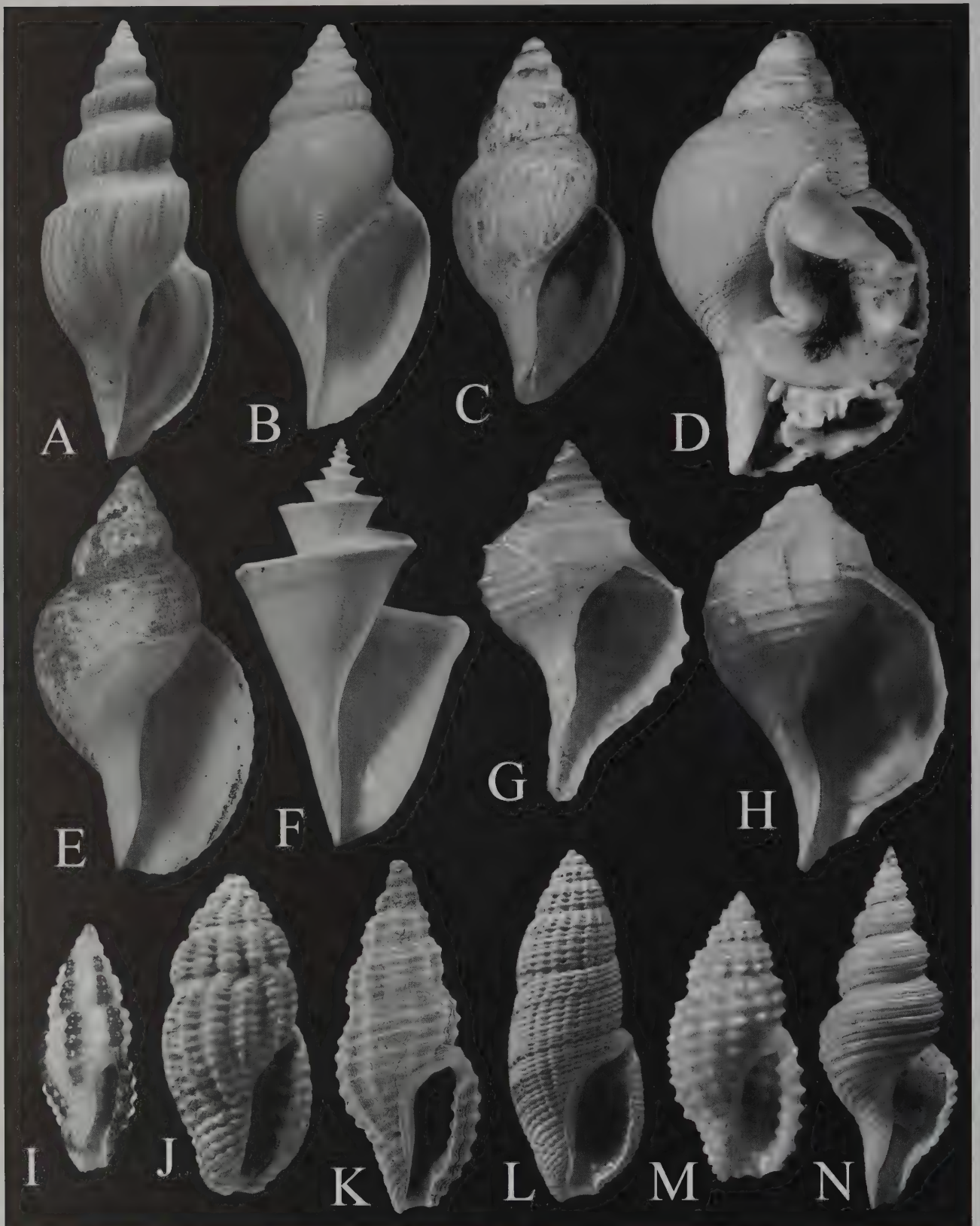
Figure 20. Shells of species described anatomically. Locality details in Table 1. Shell heights (mm). **A,** *Gymnobela pyrrhogramma* (Dautzenberg & Fischer, 1896), 20.9mm. **B,** *Gymnobela emertoni* (Verrill & Smith, 1884), 25.3mm. **C,** *Gymnobela* sp. unnamed. see Kantor & Sysoev. 1996, 26.6mm. **D,** *Phymorhynchus wareni* Sysoev & Kantor, 1995, 56.3mm. **E,**. *Phymorhynchus moscalevi* Sysoev & Kantor, 1995, 32.1mm. **F,** *Thatcheria mirabilis* Angas, 1877, 82.2mm. **G,** *Teretiopsis levicarinatus* Kantor & Sysoev, 1989, 10.7mm. **H,** *Teretiopsis abyssalis* Kantor & Sysoev, 1989, 14.3mm. **I,** *Kermia barnardi* (Brazier, 1876), 4.8mm. **J,** *Hemilienardia malleti* (Récluz, 1852), 4.8mm. **K,** *Paramontana rufozonata* (Angas, 1877), 4.5mm. **L,** *Hemidaphne reeveana* (Deshayes, 1863), 6.6mm. **M,** *Pseudodaphnella granocostata* (Reeve, 1846),11.9mm. **N,** *Teretia teres* (Forbes, 1844), 11.5mm.

of the venom gland is usually associated with the elongation of the oesophagus between the buccal mass and the nerve ring, forming the oesophageal loop. This loop allows forward movement of the buccal mass on protraction of the proboscis. Nevertheless, in at least two species of Crassispirinae with the modified gland (*Antiguraleus morganus* and *Haedropleura septangularis*) (KANTOR *et al.* 1997) and in both the above-mentioned raphitomines the oesophageal loop is absent.

Only in *Phymorhynchus moscalevi* and *Gymnobela* sp. did we observe an accessory salivary gland. The presence of accessory salivary gland(s) is sporadic within conoideans. They have been recorded in a few species of Terebridae, Turridae (subfamily Cochlespirinae) and Conidae (subfamilies Coninae, Clathurellinae and Raphitominae) (TAYLOR, 1990; TAYLOR *et al.* 1993). Although in Muricidae the gland has been shown to secrete serotonin (ANDREWS *et al.*, 1991) the function in conoideans is unknown.

An outstanding character found in raphitomines is the valve situated just posterior to the buccal cavity. So far, we have observed it in only two species, *Kermia barnardi* and *Paramotana rufozonata*. This valve resembles the valve of Leiblein, found in Rachiglossa and Nematoglossa (GRAHAM, 1941; 1966). ROBINSON (1960) supposed, that in *Mangelia brachystoma* the sphincter, separating the buccal cavity and oesophagus probably represents the vestiges of the valve (pharynx) of Leiblein. This idea was rejected by SMITH (1967a), his argument mostly based on differences in the position of the radular sac in some turrids compared with the Rachiglossa (well anterior to the valve of Leiblein). This objection can now be discounted following the discovery of numerous types of foregut arrangement in the Conoidea (TAYLOR *et al.*, 1993; KANTOR *et al.*, 1997). This point of view was supported by PONDER (1974), who also stated, that the valve may never have evolved past the oesophageal pouch stage in the toxoglossans. Finally, KANTOR (1996) even suggested that the valve of Leiblein may have originated independently twice within the Neogastropoda.

Arguments for the possible homology of the valve in Raphitominae with the valve of Leiblein in Rachiglossa concern the position of the valve just in front of the circum-oesophageal nerve ring as is found in other neogastropods and also the presence of the ciliated cone in both groups. The valve in Raphitominae differs in some respects from that of most Rachiglossa, principally in the absence of the pad of glandular cells at the base of the ciliated cone. Nevertheless, the description of the valve in Nematoglossa (= Cancellarioidei) (*Cancellaria*, GRAHAM, 1966), a possible sister group of the Conoidea (TAYLOR & MORRIS, 1988; KANTOR, 1996), resembles the valve of the raphitomines. Moreover, both raphitomine species, in which the valve was found are minute, with a shell length of only several µm, with the valve itself only about 0.1 µm in length and formed by very few cells. This may be a reason for the simplification of the valve and the absence of the glandular pad. The venom gland opens just in front of the valve (Fig. 9C) and therefore the valve does not prevent the use of venom for the immobilisation of the prey.

Evolutionary trends and feeding mechanisms in Raphitominae

A remarkable evolutionary phenomenon seen amongst species of Raphitominae is the independent loss of different foregut structures.

The proboscis. A complete morphological set of transformations of proboscis size is found in Raphitominae. The proboscis is long, occupying nearly the entire rhynchocoel in *Kermia barnardi*, *Hemilienardia malleti* and *Philbertia purpurea*. It is reduced in size in *Thatcheria mirabilis* and *Gymnobela pyrrhogramma*. While in other species, *Pseudodaphnella granicostata*, *Gymnobela emertoni*, *Teretiopsis* spp., it is vestigial and represented only by a low fold. Finally, the proboscis is completely absent in *Raphitoma linearis* and *Abyssobela atoxica* (KANTOR & SYSOEV, 1986).

The venom apparatus. The venom apparatus is absent in *Gymnobela emertoni*, *Teretiopsis* spp., *Raphitoma linearis*, and *Abyssobela atoxica*. Hitherto, it has been supposed that the loss of venom gland was linked with a great reduction or absence of the radula (KANTOR & SYSOEV, 1989; TAYLOR *et al.*, 1993) but in *Pseudodaphnella granicostata* the venom gland persists while the radula is absent.

The radula. A radula is absent in *Pseudodaphnella granicostata*, *Teretiopsis* spp., *Raphitoma linearis*, *Abyssobela atoxica* (KANTOR & SYSOEV, 1986) and *Clathromangelia granum* (OLIVERIO, 1995).

The salivary glands. The tubular salivary glands can be paired or single (*Thatcheria mirabilis*, *Pseudodaphnella granicostata*) or completely absent as in *Teretiopsis* spp., *Raphitoma linearis* and *Abyssobela atoxica*.

Thus, it is seen, that any of the major foregut organs can be lost without relation to the others. A similar loss of foregut structures has been observed in the Terebridae (TAYLOR 1990; TAYLOR *et al.* 1993; SIMONE, 1999). Some species have a complete set of foregut organs including rhynchodeal introvert , proboscis, radula, two pairs of salivary glands, venom apparatus while other species have lost some or all of these organs. In the most derived state the animals feed suctorially using the introvert with all other major structures absent (MILLER, 1975).

The possible role of heterochrony in gastropod evolution has been advocated by LINDBERG (1988) and PONDER and LINDBERG (1997). The trends towards simplification of the foregut observed in Raphitominae may be accounted for by paedomorphosis and it is likely that such changes could have occurred in parallel in different clades. BALL, TAYLOR & ANDREWS (1997) demonstrated that during the ontogeny of *Nucella*, the acinous salivary glands of the adult develop from initial tubular ducts of the embryo. Thus, the tubular salivary glands of raphitomines (and also Mangeliinae) likely represent a paedomorphic condition. Similarly, studies of the ontogeny of the neogastropod proboscis (BALL, ANDREWS & TAYLOR 1997) suggest that the reduction or absence of the proboscis in many raphitomines may represent progressively paedomorphic states. Loss of radula and venom apparatus may also be explained by paedomorphosis. In fact the venom gland is one of the last of the major foregut organs to develop during ontogeny (A.D. Ball

personal communication). Amongst those raphitomine species possessing a radula there is considerable variety of tooth form from the elongate barbed, *Conus*-like teeth of *Phymorhynchus* to the simple awl shaped teeth of *Paramontana*. During ontogeny the teeth of *Conus* become progressively more elaborate (NYBAKKEN, 1990), the early post-metamorphic teeth being simple and awl shaped. Thus the simple forms of raphitomine teeth could represent paedomorphic states. It is tempting to suggest that the small size of many raphitomines is also a paedomorphic feature which may be associated with the simplification of the foregut. Although most of the species having the simplified foregut are indeed small there are also small species with proboscis, venom apparatus and radula.

Feeding mechanisms

The remarkable feature of the conoidean feeding mechanism and not restricted just to *Conus*, is the deployment of single radular teeth at the proboscis tip for stabbing and envenomation of the prey. It has been now been demonstrated for a wide range of different conoideans that the teeth are usually held at the proboscis tip by one or more buccal tube sphincters (KANTOR & TAYLOR, 1991; TAYLOR *et al.*, 1993), often in conjunction with an epithelial pad located at the distal end of the buccal tube. In Raphitominae, the buccal tube sphincters are present in only a few of the species possessing a proboscis; these are *Phymorhynchus* spp. and *Gymnobela pyrrhogramma*. In another species, *Hemilienardia malleti*, in which the buccal tube sphincter was absent, the tooth was found at the proboscis tip, probably held by a small pad of epithelial cells. In all other species having a radula and proboscis, the obvious structures for gripping teeth at the proboscis tip were absent. Previously, conoidean species possessing a radula but lacking buccal tube sphincters have been recorded only among Crassispirinae (Turridae), namely *Burchia spectabilis* and *Inquisitor latifasciata* (KANTOR *et al.*, 1997). This absence of a sphincter suggests that teeth are not held at the proboscis tip and therefore no stabbing occurs during prey capture.

The diet of Raphitominae is poorly known. In the stomach of one specimen of *Gymnobela subaraneosa* (DAUTZENBERG & FISCHER, 1896) the radula of the rissoid gastropod *Benthonella tenella* was found (BOUCHET & WARÉN, 1980). An undescribed species *Phymorrhynchus* from the East Pacific Rise was reported with fragments of the gastropod *Neomphalus fretterae* in its gut (Warén & Bouchet, 1989), whilst it has also been observed, that *Phymorrhynchus moscalevi*, living on the hydrothermal vents, feeds on the bivalve *Bathymodiolus* (A. Warén, personal communication). The food being at least partially digested within the rhynchocoel. Because of this paucity of feeding information, our analysis of the possible feeding mechanisms of the Raphitominae is therefore based on the morphology of the foregut and by comparison with other conoideans where the feeding process is better known. Morphological evidence suggests that there are at least three types of feeding mechanism among Raphitominae.

The normal toxoglossan type (Feeding mechanism type 3 of TAYLOR *et al.*, 1993).

The species belonging to this group probably all use marginal teeth at the proboscis tip for stabbing and envenomation of prey. These include amongst the raphitomine species we examined; *Gymnobela* sp., *Gymnobela pyrrhogramma, Phymorhynchus* spp., *Hemilienardia malleti* and probably *Paramontana rufozonata*. In all these species the proboscis and venom apparatus are well developed and there are epithelial pads and sometimes sphincters in the buccal tube for holding radular teeth near the proboscis tip. It is possible that *Philbertia purpurea* belongs to this group as well because according to the drawing of the foregut (SHERIDAN *et al.*, 1973), its anatomy is very similar to that of *Hemilienardia malleti*. Although *Paramontana rufozonata* lacks a buccal tube sphincter or epithelial pad, the mouth opening is very narrow and is surrounded by a circular fold. This fold may be used for holding a radular tooth at the proboscis tip.

Envenomation of the prey without radular stabbing.

Species belonging to this group including *Hemidaphne reeveana*, *Thatcheria mirabilis*, *Pseudodaphnella granicostata*, *Philbertia leufroyi boothi*, either lack a radula but possess a venom gland (*P. granicostata*) or lack any mechanisms for holding radular teeth at the proboscis tip. Thus, in *T. mirabilis* and *D. reeveana* the mouth opening is very wide and without sphincters or an epithelial pad. In *Philbertia leufroyi boothi*, although both radular and venom apparatus are present, the proboscis is vestigial and incapable of holding teeth (SMITH, 1967). It is also possible that *Kermia barnardi* also belongs to this group for its buccal tube also lacks any obvious mechanisms for holding teeth. It is suggested that these animals either envenomate their prey after swallowing or somehow immobilise the prey by squirting venom.

Capture of prey without stabbing and envenomation.

Species, belonging to this group either lack proboscis, radula and venom apparatus (*Teretiopsis* spp., *Raphitoma linearis*, *Clathromangelia granum*,*Abyssobella atoxica*), or, like *Gymnobela emertoni* possess only a vestigial radula. In all these species, there is well-developed cavity between the rhynchodaeum and the body wall and prominent radial muscles cross this cavity. KANTOR & SYSOEV (1989) have proposed previously that contraction of the radial muscles increases the inner volume of the rhynchocoel and causes negative pressure within it. This facilitates a suctorial engulfment of the prey into the rhynchocoel.

The rhynchostomal introvert is present in some species classified into each of the three groups. It likely has a role in prey capture and manipulation. By comparison, in many species of Terebridae the rhynchostomal introvert has taken over the role of the proboscis in prey capture and become the main feeding organ (MILLER, 1975).

Comparison of Raphitominae with other subfamilies of Conidae

Amongst the Conidae, Raphitominae are closest in morphology to Mangeliinae and Coninae. Both Mangeliinae and

raphitomines possess tubular rather than the more usual acinous type of salivary glands. Tubular glands are characteristic for both subfamilies but otherwise are found only in a few Crassispirinae species (KANTOR *et al.*, 1997) The two subfamilies also share a rather similar morphology of the muscular bulb of the venom gland. While in Raphitominae the bulb is single-layered, in Mangeliinae the wall of the bulb has two layers, but the outer is extremely thin and is formed by only a single sheet of muscle fibres.

In this connection the position of *Gymnobela* sp. should be discussed in more detail. This still unnamed species was attributed to Raphitominae on the basis of similarity of general shell shape with some representatives of *Gymnobela* (KANTOR & SYSOEV, 1996). Protoconchs of all available specimens were corroded and therefore its sculpture (which is characteristically diagonally cancellate in *Gymnobela*) is unknown. The anatomy of this species is very unusual within the Raphitominae (e.g. very long proboscis, radular sac without radular teeth), while the muscular bulb of the venom gland is very similar to that of Mangeliinae (still very poorly studied anatomically) in having characteristic very thin outer layer, formed by a single sheet of muscle fibres. Taking this into consideration we suggest, that this species should be transferred to Mangeliinae.

When comparing Raphitominae with Coninae, there are some striking similarities between *Conus* species and some *Gymnobela* species. The characters that are shared by species of both subfamilies are the presence of the septum (which in *Conus* can be both basal as in *Conus ventricosus*, Fig. 16, or situated in the middle sector of the rhynchocoel as in *Conus boholensis*, Fig. 17; both types of arrangement are found in Raphitominae) and additionally, the shape and size of the proboscis (compare *Gymnobela pyrrhogramma*, Fig. 1 and *Conus* spp., Figs. 16, 17). The main differences are the acinous salivary glands in *Conus* compared with tubular salivary glands in Raphitominae and the multi-layered muscular bulb of *Conus* species. Both these character states represent plesiomorphic conditions, found in most groups of Turridae and Conidae. The cladistic analysis also supposes that Coninae are the sister group of Raphitominae

CONCLUSIONS

This study confirms our initial impression that the foregut of the Raphitominae exhibits more structural variation than any other conoidean family or subfamily excepting the Terebridae. The range of variation extends from species which possess a full complement of conoidean foregut organs (proboscis, venom apparatus, salivary glands, radula) to those in which most or all of these structures are absent. Despite this variation in foregut configuration, monophyly of the Raphitominae is supported by several apomorphies such as the cancellate protoconch, the single layer to the muscular bulb and the rhynchodeal septum. The division of conoidean foreguts into just two categories, the intraembolic and polyembolic types (SMITH, 1967a) is a gross oversimplification. As more and more conoidean taxa are studied anatomically, for example the Crassispirinae (KANTOR *et al.* 1997), Cochlespirinae (MEDINSKAYA ,1999) or Terebridae (TAYLOR, 1990; SIMONE 1999), an extraordinary disparity of foregut configurations is being revealed which presumably reflects a great diversity of feeding behaviours and prey capture mechanisms. Unfortunately, little information is available on the biology of these animals.

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Reproduction and larval development of the *Strombina*-group (Buccinoidea: Columbellidae) and related gastropods: testing the use of the larval shell for inference of development in fossil species

Helena Fortunato

KEY WORDS: *Strombina*-group, neogastropods, protoconch morphology, inference, fossil species

ABSTRACT The primitive planktotrophic mode of larval development of gastropods of the *Strombina*-group was replaced during the closure of the Isthmus of Panama by predominantly direct development in the Caribbean, but remains the predominant mode in the eastern Pacific. As with other gastropods, the inference of larval development in this group is based on the morphology of the larval shell, but the validity of this method has not been tested by direct biological observations. Reproduction was observed for six species of the *Strombina*-group and compared to those for two other species reported previously. These data were then used to infer the mode of development for four additional species for which only preserved egg capsules and eggs were available. In all 10 cases, the results agree with the mode of development inferred on the basis of larval shell dimensions and numbers of whorls.

RIASSUNTO Il tipo primitivo di sviluppo larvale (planctotrofico) nei gasteropodi dello *Strombina*-group è stato sostituito durante la chiusura dell'Istmo di Panama da uno sviluppo predominantemente diretto nei Caraibi, mentre è rimasto il tipo di sviluppo prevalente nel Pacifico orientale. Come in altri gasteropodi, l'inferenza del tipo di sviluppo in esemplari di questo gruppo è basata sulla morfologia della conchiglia larvale, ma la validità del metodo non è stata verificata per mezzo di osservazioni biologiche dirette. La riproduzione è stata osservata in sei specie dello *Strombina*-group e comparata con quella di due altre specie studiate precedentemente. Tali dati sono stati usati per inferire il tipo di sviluppo in altre quattro specie per le quali erano disponibili solo dati su uova e capsule ovigere. In tutti i 10 casi, i risultati sono in accordo con il modo di sviluppo inferito sulla base della dimensioni e del numero di giri della conchiglia larvale.

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INTRODUCTION

The *Strombina*-group sensu Jung (1989) is a highly diverse group and appear as extremely well preserved fossils throughout Neogene and Quaternary deposits of Tropical America. Extensively collected, and with their taxonomy recently revised (Jung, 1989), species of this group have been used as a model system to study temporal and spatial evolutionary consequences of the Pliocene rise of the Isthmus of Panama (Jackson *et al.*, 1993; Jackson, 1994; Jackson *et al.*, 1996).

Inferences based mainly on the morphology of the protoconch of both fossil and living species of this group indicate that the oldest species (Early and Middle Miocene) were entirely planktotrophic (Jackson *et al.*, 1996). The proportion of non-planktotrophs increased thereafter, but the nature of this change was reversed between the two oceans. Most younger species in the Caribbean exhibited direct development whereas planktotrophic development prevailed in the eastern Pacific with lecithotrophic and direct development combined of roughly equal secondary importance. These striking shifts in the life history of the *Strombina*-group can be used to model the relationships between environmental events and processes of speciation and extinction. In order to do this, however, we need to calibrate modes of development inferred through shell morphology by comparison with the life histories of living species based on observations of larval development.

Gastropod development can be divided into groups based on the number and size of eggs produced, the mode of larval nutrition, and whether later stages of larval development occur within the egg capsule or in the plankton (Thorson, 1946, 1950, 1961; Mileikovšky, 1971; Jablonski & Lutz, 1983; Jablonski, 1986; Lima & Lutz, 1990). Planktotrophic species lay many small capsules filled with small embryos and without any extra food resources. These embryos develop into veliger larvae, usually with a well developed velum and digestive organs. They swim and feed actively in the plankton where they commonly pass a relatively large period of time before settlement and metamorphosis. In contrast, non-planktotrophic species lay a small number of large capsules containing a few large embryos that may exhibit one of two different modes of development. In the first case, commonly termed direct development, the veliger stage and metamorphosis take place inside the capsule where the embryos develop into fully competent juveniles that crawl away. Direct development is commonly associated with the production of extra nutritive eggs and cannibalism of more slowly developing embryos. In the second case, termed lecithotrophic development, the embryo develops into a 'pediveliger' larva with a small velum and a definitive foot. 'Pediveligers' live in the plankton for a short period of time without feeding, before settling as juveniles.

Table 1. Summary of reproductive aspects for 12 species of the *Strombina*-group and related gastropods.

	Egg Capsule Length (mm)	Egg Capsule Width (mm)	Egg Capsule Aperture Length (mm)	Egg Capsule Aperture Width (mm)	No. Eggs/ Capsule	Egg Diameter (µm)	No. Embryos/ Egg Capsule	Shell Length at Hatching (µm)	Hatching Stage
EASTERN PACIFIC SPECIES									
1- *Bifurcium bicanaliferum*[a]	1.0 ± 0.1 (n = 27)	0.8 ± 0.1 (n = 27)	0.3 ± 0.01 (n = 27)	0.2 ± 0.02 (n = 27)	22 ± 2 (n = 24)	151 ± 11 (n = 30)	20 ± 3 (n = 29)	234 ± 10 (n = 40)	veliger 1.5 whorls
2-*Sincola gibberula*	1.1 ± 0.1 (n = 25)	1.0 ± 0.03 (n = 25)	0.3 ± 0.01 (n = 12)	0.2 ± 0.01 (n = 12)	25 ± 2 (n = 25)	150 ± 12 (n = 25)	21 ± 2 (n = 17)	270 ± 10 (n = 40)	veliger 1.75 whorls
3-*Sincola sinuata*	1.5 ± 0.2 (n = 25)	1.3 ± 0.2 (n = 25)	0.4 ± 0.05 (n = 25)	0.3 ± 0.04 (n = 25)	ND	ND	ND	ND	ND
4-*Strombina elegans*	3.5 ± 0.3 (n = 25)	3.2 ± 0.3 (n = 25)	0.9 ± 0.2 (n = 25)	0.5 ± 0.1 (n = 25)	56 ± 21 (n = 11)	304 ± 20 (n = 30)	ND	400 ± 73 (=17)	veliger 1.25 whorls
5-*Strombina lanceolata*	2.1 ± 0.08 (n = 25)	1.7 ± 0.09 (n = 25)	0.5 ± 0.04 (n = 25)	0.4 ± 0.05 (n = 25)	30 ± 7 (n = 20)	336 ± 30 (n = 44)	ND	ND	ND
6-*Strombina recurva*	2.4 ± 0.1 (n = 22)	2.0 ± 0.3 (n = 22)	0.6 ± 0.05 (n = 22)	0.5 ± 0.07 (n = 22)	27 ± 7 (n = 8)	396 ± 75 (n = 21)	14 ± 3 (n = 9)	423 ± 82[c] (n = 15)	veliger[c] 1.75 whorls
7-*Clavistrombina clavulus*	2.2 ± 0.2 (n = 26)	1.7 ± 0.2 (n = 26)	0.7 ± 0.08 (n = 26)	0.6 ± 0.04 (n = 26)	14 ± 2 (n = 26)	315 ± 31 (n = 58)	ND	470 ± 25 (n = 15)	veliger 1.5 whorls
8-*Cosmioconcha modesta*	1.9 ± 0.2 (n = 24)	1.5 ± 0.2 (n = 24)	0.5 ± 0.08 (n = 24)	0.4 ± 0.05 (n = 24)	22 ± 5 (n = 16)	211 ± 27 (n = 25)	17 ± 3 (n = 25)	312 ± 16 (n = 25)	veliger 1.75 whorls
9-*Cosmioconcha parvula*	1.3 ± 0.1 (n = 22)	1.1 ± 0.1 (n = 22)	0.3 ± 0.07 (n = 22)	0.3 ± 0.06 (n = 22)	15 ± 2 (n = 17)	209 ± 28 (n = 25)	4 (n = 12)	284 ± 32[c] (n = 15)	veliger[c] 1.25 whorls
10- *Cosmiconcha redheri*	0.6 ± 0.07 (n = 13)	0.5 ± 0.04 (n = 13)	0.3 ± 0.05 (n = 13)	0.2 ± 0.05 (n = 13)	ND	ND	ND	ND	ND
CARIBEAN SPECIES									
11- *Strombina pumilio*[b]	2.1-2.4	2.0-2.1	1.3	1.0-1.1	5	616 ± 48	5	947 ± 97	crawling juvenile 1.25-1.5 whorls
12- *Strombina francesae*[b]	2.4 ± 0.3	2.1 ± 0.2	1.3 ± 0.3	0.9 ± 0.1	5	571 ± 35 (n = 22)	5	900 ± 46	crawling juvenile 1.25-1.5 whorls

ND — No data available
a — data from FORTUNATO *et al.*, 1998
b — data from CIPRIANI & PENCHASZADEH, 1993
c — intracapsular veliger

The last decade witnessed an increased interest in the study of the relation between larval ecology and species longevity (HANSEN, 1978; VALENTINE & JABLONSKI, 1986; GILI & MARTINELL, 1994; OLIVERIO, 1996a, 1996b), dispersal capacity (BOUCHET 1981; BOUCHET & WAREN, 1979; 1994; GALLARDO & PERRON, 1982; LEAL & BOUCHET, 1991; OLIVERIO, 1994) and the utility of protoconch morphology in systematic studies (BOUCHET, 1990; OLIVERIO & TRINGALI, 1992; OLIVERIO, 1995). Most of these works deal with wide distributed groups like nassarids and turrids. Despite several studies on the Columbellidae (PETIT & RISBEC, 1929; THORSON, 1940; FRANC, 1941; KNUDSEN, 1950; AMIO, 1955; MARCUS & MARCUS, 1962; SCHELTEMA & SCHELTEMA, 1963; SCHELTEMA, 1969; D'ASARO, 1970; BANDEL, 1974; FLORES, 1978), very little is known about the development of the species of the *Strombina*-group. JUNG (1989) commented on the occurrence of egg masses on adults of several species, but the first direct observations of reproduction were reported by CIPRIANI & PENCHASZADEH (1993) on two Caribbean species, *Strombina pumilio* (Reeve), 1858, and *S. francesae* J. Gibson-Smith, 1974. I reported on the reproductive modes of two eastern Pacific species, *Bifurcium bicanaliferum* (B. G. Sowerby I, 1832) and *Sincola gibberula* (G. B. Sowerby I, 1832) (FORTUNATO, 1995; FORTUNATO *ET AL.*, 1995; FORTUNATO *ET AL.*, 1998). The purpose of this paper is to present new data on several other eastern Pacific species of the *Strombina*-group and related columbellids, as well as summarize the scattered information given in previous reports.

MATERIALS AND METHODS

Observations were made on seven species of the *Strombina*-group sensu Jung (1989) and three species of the genus *Cosmioconcha* Dall (1913). Identifications follow JUNG (1989) for species of the *Strombina*-group, and KEEN (1971) as modified by RADWIN (1977a, 1977b, 1978) for the genus *Cosmioconcha*.

Adults, masses of egg capsules and juveniles were collected at several localities in the Gulf of Panama (Fig. 1: stn.1-3) from 1994 through 1996, and in the Gulf of Chiriqui in 1995 and 1997 (Fig.1: stn. 4). Collections were made both by hand along the

Table 2. Summary for modes of development based on direct biological observations, inferred from capsular and larval morphology, and protoconch morphology for 12 species of the *Strombina*-group and related gastropods.

	Type of Larvae	Biological Observations No. Eggs/ Capsule	No. Eggs/ Size Capsule	No. Surviving Embryos/Capsule	Size Aperture /Size Capsule	Protoconch Morphology Max Diameter (?m)	Max Number of Volutions
EASTERN PACIFIC SPECIES							
1- *Bifurcium bicanaliferum*[a]	P	22	22	20	0.3	354	2.5
2- *Sincola gibberula*	P	25	22.7	21	0.3	410	3.0
3- *Sincola sinuata*	(P)	ND	ND	ND	0.3	516	3.0
4- *Strombina elegans*	P	56	16	ND	0.3	548	3.0
5- *Strombina lanceolata*	(P)	30	14.3	ND	0.2	505	2.75
6- *Strombina recurva*	(P/L)	27	11.3	14	0.3	465	2.25
7- *Clavistrombina clavulus*	P	14	6.4	ND	0.3	458	3.0
8- *Cosmioconcha modesta*	P	22	11.6	17	0.3	670	2.5
9- *Cosmioconcha parvula*	P	15	11.5	4	0.2	514	2.5
10- *Cosmioconcha redheri*	(P)	ND	ND	ND	0.5	700	3.0
CARIBEAN SPECIES							
11- *Strombina pumilio*[b]	D	5	2.3	5	0.6	660	1.5
12- *Strombina francesae*[b]	D	5	2.3	5	0.5	483	1.5

ND — No data available

a — data from FORTUNATO *et al*., 1998

b — data from CIPRIANI & PENCHASZADEH, 1993

intertidal zone at low tides, and by dredging offshore. Animals were brought to the Naos Marine Laboratory of the Smithsonian Tropical Research Institute (STRI) in Panama, where they were kept in 15 l outside aquaria with running and aerated sea water. Adults were regularly fed fish scraps. Water temperature was not regulated in any way: aquaria were kept always outside and water was taken directly from the ocean. Observations were made on egg masses laid both in the field and in the laboratory. After hatching, larvae were kept in separated aquaria within the same water system as the adults. No extra food was provided for the larvae.

Measurements and counts of capsules, eggs, embryos, and veligers were made using a Wild M7 stereoscopic microscope. Observations were made on both living and fixed material (3% seawater buffered formaldehyde). A scanning electron microscope EOL 5300LV was used to photograph egg capsules and veliger shells which were cleaned with filtered sea water and kept in a 70% ethanol solution before being coated with gold. Adult specimens, egg capsules and veliger shells of all the species described here were deposited at the Field Museum of Natural History (FMNH), Chicago, at the Academy of Natural Sciences of Philadelphia (ANSP), and at the Muséum d'Histoire Naturelle de Genève (MHNG).

Figure 1. Map of Panama showing the localities where collections where made. Gulf of Panama: Bique, Venado, Farfan, Taboga Is., Taboguilla Is., Las Perlas archipelago. Gulf of Chiriqui: Pt. San Pedro, Uva Is., Secas Is.
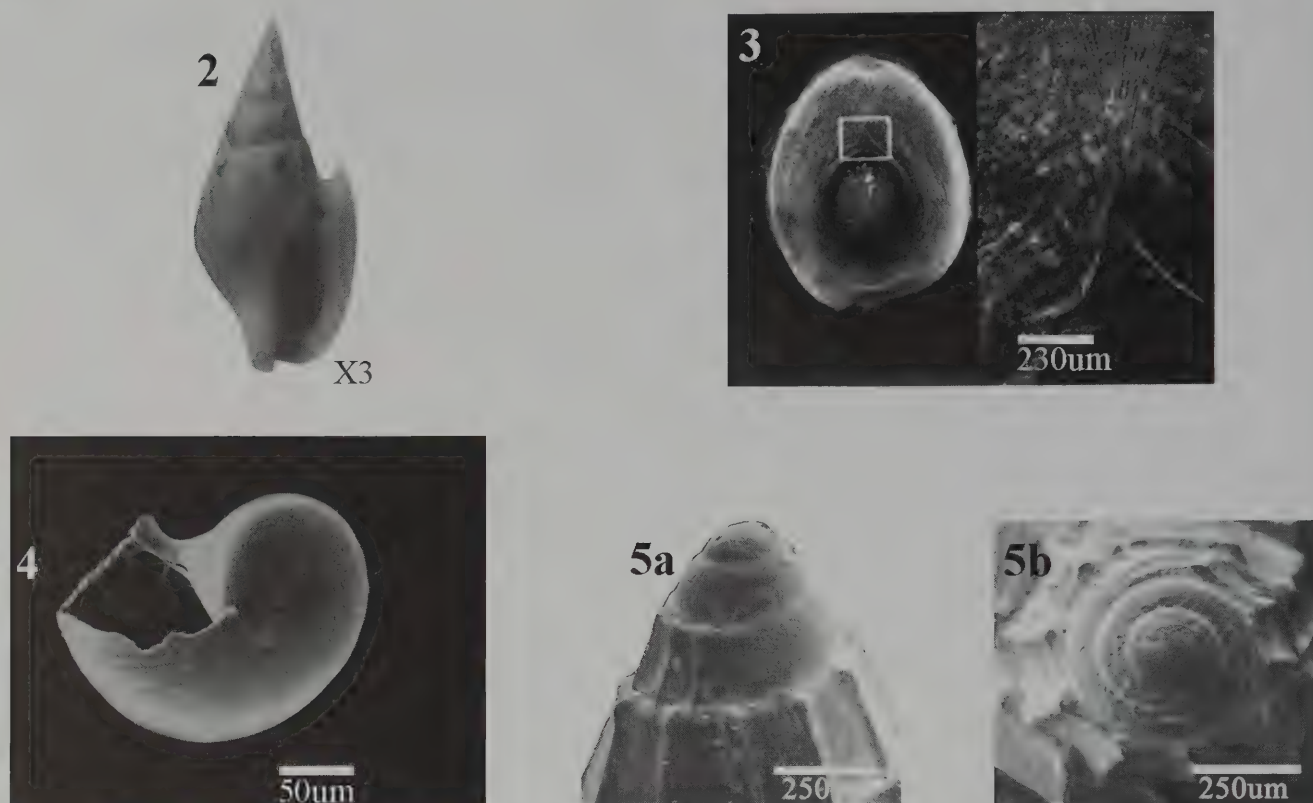
Figure 2-5. *Bifurcium bicanaliferum* (B. G. Sowerby I, 1832) - Fig. 2: adult specimen (from JUNG, 1989 with permission); Fig. 3: SEM picture of the egg capsule (FMNH293337). The right side is a close up of the surface of the enclosed area in the left; Fig. 4: SEM picture of the veliger shell (FMNH293337); Fig. 5a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).
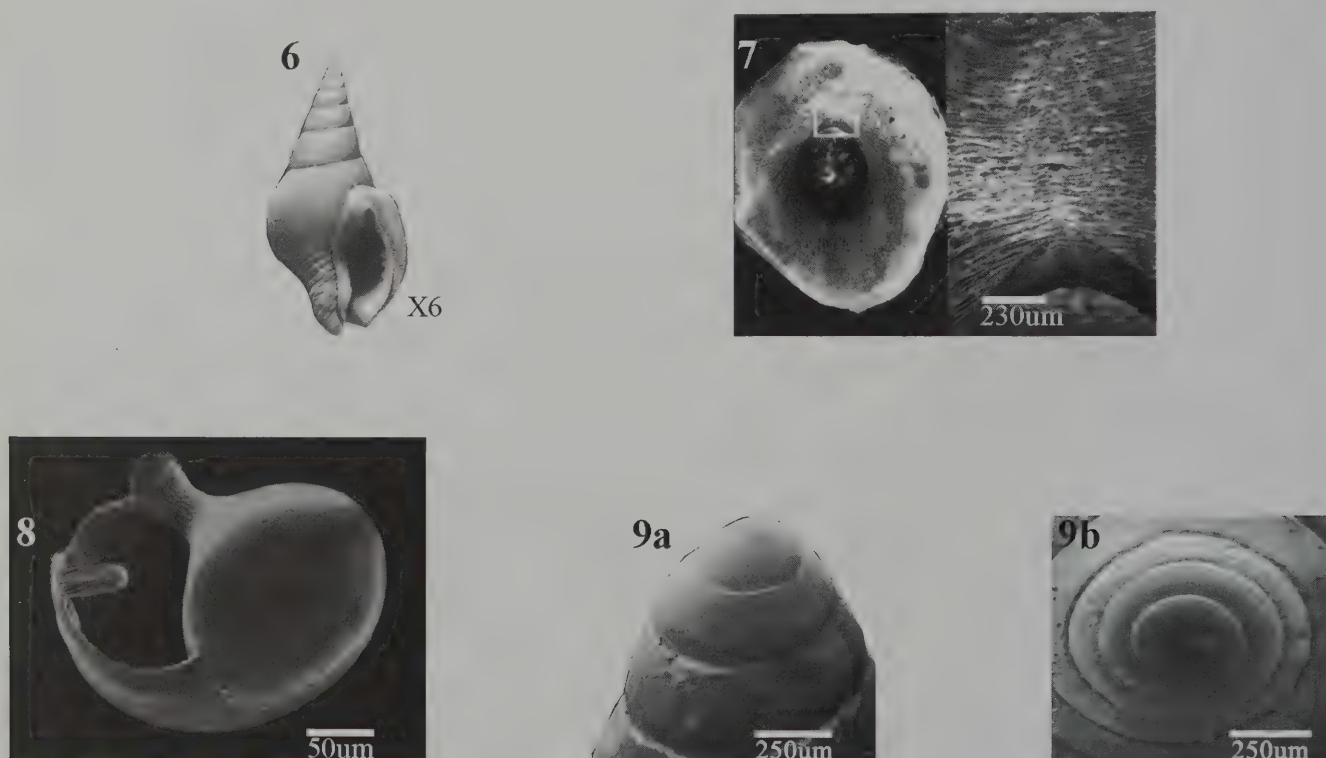
Figure 6-9. *Sincola (Dorsina) gibberula* (B. G. Sowerby I, 1832) - Fig. 6: adult specimen (from JUNG, 1989 with permission); Fig. 7: SEM picture of the egg capsule (FMNH293338). The right side is a close up of the surface of the enclosed area in the left; Fig 8: SEM picture of the veliger shell (FMNH293338); Fig. 9a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).

RESULTS

Measurements and counts for all 12 studied species are summarized in Table 1. The biological observations and protoconch morphological parameters used to infer modes of development are presented in Table 2. I first describe my own results from the eastern Pacific and then compare these to earlier results from the Caribbean.

I - Eastern Pacific Species

Bifurcium bicanaliferum **(B. G. Sowerby I, 1832)**

Bifurcium bicanaliferum (Fig. 2) is the only living species of this genus, which originated in the Caribbean Sea during the Miocene. Live adults and juveniles were collected during the dry season, January through April of 1994-1996, from extensive mud flats exposed during low tides at Bique and Venado beaches, in the Gulf of Panama (Fig. 1: stn. 3). They were also dredged from muddy sands near Taboga and Taboguilla islands, and in the Las Perlas archipelago (Fig. 1: stn. 1-2). Animals were usually found in aggregations of up to 30 specimens, gliding with their extremely long foot over the surface of the mud or buried in the top 2-3 cm. The only available hard substrate for oviposition was the shells of conspecifics. No oviposition was observed in the laboratory. Capsules are dome shaped with a basal membrane and an almost circular escape aperture in the center (Fig. 3). A suture runs through the center of the capsule, almost dividing it in two. This suture is most noticeable from the side where the escape aperture is deformed. Intracapsular development was completed 15 days after collection. Free swimming veligers at hatching have a well developed velum with black spots at the base of the cilia and a shell comprising 1.5 whorls (Fig. 4). Through the shell, the heart, the digestive gland, the stomach, and branchia are visible. Observations of the larvae showed the presence of greenish filamentous microalgae in the guts, possibly ingested as food. Veligers are very active and usually remain near the surface of the water. They survived for about 15 days, after which they all died. For this reason, further development and metamorphosis could not be observed. The hatching of swimming and feeding veliger larvae, the sinusigerous outer lip of the protoconch, and the fact that the larval shell has 2½ volutions (Fig. 5a, b) at the moment of metamorphosis indicates that growth takes place during the swimming stage, and that this species should have planktotrophic development.

Sincola (Dorsina) gibberula **(G. B. Sowerby I, 1832)**

Sincola gibberula (Fig. 6) is one of three living species of this once diverse genus in Neogene deposits of the Caribbean and eastern Pacific. Live adults were dredged during the dry season (January through March) of 1995 at Taboga, Tabogilla, and the Las Perlas archipelago in the Gulf of Panama (Fig.1: stn. 1-2). Animals were usually found alone or in small groups of 2-3 specimens in fine muddy sand. About 20 adult animals with compact masses of eggs attached to their shells were kept in the laboratory for observation. No oviposition was noticed in captivity.

Adults lay capsules in compact rows along the dorsal side of a congeneric shell, often covering the shell almost completely. Capsules were yellowish, translucent, and hemispheric, with a short and irregular basal membrane. The almost circular escape aperture, covered by a thin lid, is located in the upper side of the capsule (Fig. 7). A suture that nearly divides the capsule into two halves can be observed extending from the deformed escape aperture to the basal membrane. Successive layers of capsules commonly obstruct the escape aperture of capsules deposited below, causing mortality of the embryos within. Uncleaved zygotes, as well as embryos in different developmental stages were found in the same egg mass. Eggs were round and yellowish (Fig. 10A).

Intracapsular development takes place within about 15 days. The trochophore has two velar lobes (Fig. 10B, C). The veliger prior to hatching has a thin, fragile, transparent shell with no siphonal canal (Fig. 10D). Larvae increase their activity the day prior to hatching. The hatching veliger bears a shell and a large bilobate ciliated velum at the anterior end with black spots at the base of the cilia. The veliger shell has a short siphonal canal with a reddish edge (Fig. 8) and a small, round operculum. Through the shell, the heart, the digestive gland, the stomach, and branchia are visible. This free-swimming larval stage is very active and usually remains near the surface of the water. Veligers were alive for about 15-17 days. Although no extra food was provided, observations of the larvae showed the presence of greenish filamentous microalgae in the guts. The transition from the planktonic stage to the crawling juvenile was not observed because all the veligers died within 17 days after hatching. The hatching of swimming and feeding veliger larvae, and the sinusigerous shape of the protoconch outer lip are strong evidence for planktotrophic development. On the other hand, the larval shell has over 3 volutions (Fig. 9a, b) at the time of metamorphosis, which also indicates a period of growth during the planktonic stage.

Sincola (Sinuina) sinuata **(G. B. Sowerby II, 1874)**

Two adult specimens of *Sincola sinuata* (Fig. 11) were collected in March 1997 while dredging at Uva Island, Gulf of Chiriqui (Fig. 1: stn. 4) at a depth of 18 m. The substrate was fine muddy sand. Both animals were covered with a layer of transparent, almost spherical capsules with a well defined sutural ridge along the middle of the capsule and a comparatively wide basal flange (Fig. 12). A circular escape aperture is located in the center of the capsule. No eggs or larval shells were found in any of the collected material. The protoconch (Fig. 13a, b) has a weakly sigmoid outer lip and 3 volutions at the time of metamorphosis.

Strombina (Spiralta) elegans **(G. B. Sowerby I, 1832)**

Strombina elegans (Fig. 14) is one of two living species of this subgenus. Several live adults were collected in July 1995 by dredging on coarse to fine coralline sand near Uva Island (Fig. 1: stn. 4) at a depth of 20-40 m. One of the animals spawned on July 16. Ten egg capsules were laid on the walls
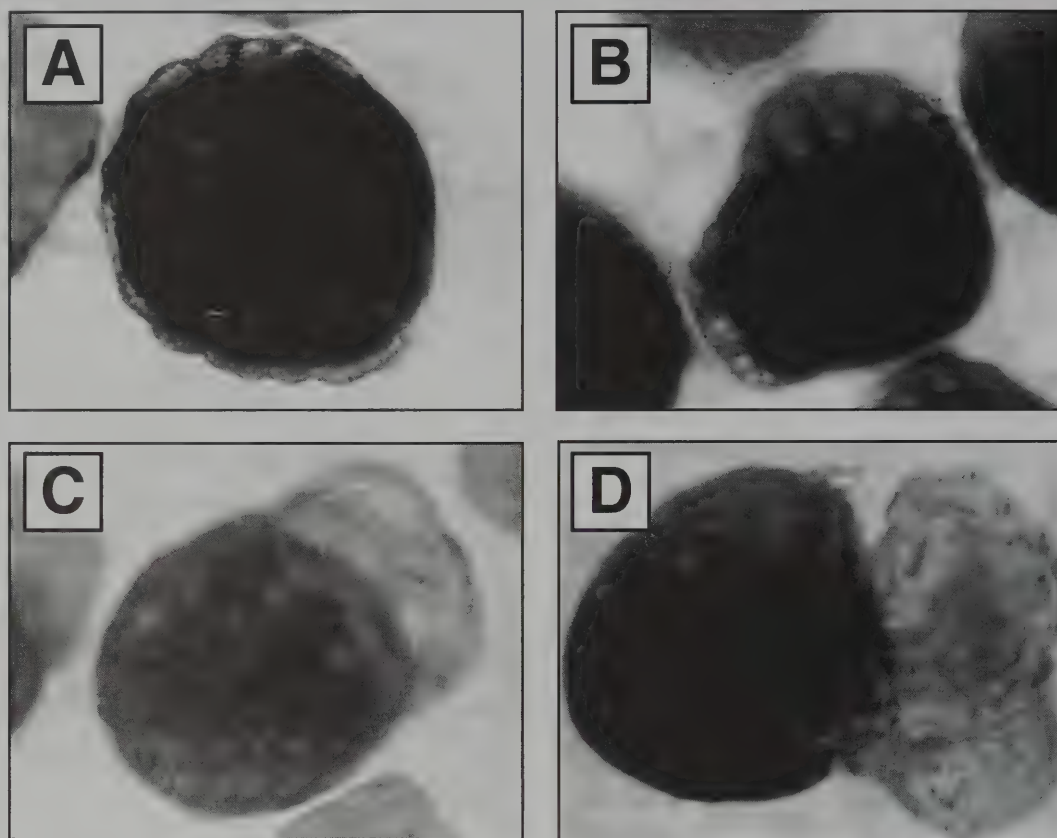
Figure 10a-d. Stages of the embryonic development of *Sincola gibberula* - A: eggs; B: embryo without torsion; C: embryo after torsion, velar lobes start to develop; D: pre-hatching veliger. Notice the thin shell and the velar lobes.

Figure 11-13. *Sincola (Sinuina) sinuata* (B. G. Sowerby II, 1874) - Fig. 11: adult specimen (from JUNG, 1989 with permission); Fig. 12: SEM picture of the egg capsule (FMNH293339). The right side is a close up of the surface of the enclosed area in the left; Fig. 13a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).

of the container where the animals had been placed just after collection. This was the first observation of spawning by a species of this group in captivity. On July 26, 20 egg capsules were observed on the walls of the aquaria where the animals were kept at the STRI Naos Marine Laboratory. Such behavior seems to be unusual among the species observed which otherwise seemed to prefer to lay their eggs upon conspecific shells. Capsules are semispherical, yellowish and have a thin but well delineated basal membrane just above the concave basal wall (Fig. 15). A suture runs longitudinally from one side of the egg capsule to the other. The capsules are the largest of all the species studied but the central, ellipsoidal escape aperture is comparatively very small (Table 1). Capsules were measured and removed to another aquarium for observation. Intracapsular development was very rapid. Embryos underwent torsion in four days. Intracapsular veligers with a very fragile shell were observed on the seventh day and oclusion occurred on the 9th. Free swimming veligers were very small at hatching, with a small velum and a shell comprising 1.25 whorls (Fig. 16). All the veligers died within 5 days of hatching. No signs of feeding were observed during the period that the larvae were alive. Nevertheless, the sinusigerous shape of the protoconch outer lip and the fact that the larval shell has 3 volutions (Fig. 17a, b) at the time of metamorphosis indicates that growth occurs during the veliger phase, i.e. it should be a planktotrophic larva.

Strombina (Strombina) lanceolata **(G. B. Sowerby I, 1832)**
Strombina lanceolata (Fig. 18a, b) is known only from the Galapagos Islands where it is relatively abundant (Finet 1985, 1991, 1994). It's habitat ranges from fine coral sand through hard coral mud, coralline rubble, fine sand, coarse sand, and mud. Jung (1989, p. 60, fig. 82:19-21) found several egg capsules attached to a specimen in a sample from the San Diego Museum of Natural History collected by dredging on a sandy bottom in Tagus Cove, Albermarle island. I also had access to material collected by Dr.Yves Finet in 1993 in Gardner Bay, Española Is., and in Post Office Bay, Floreana Is. Capsules are roundish, with the basal membrane and the ellipsoid escape aperture characteristic of other studied members of the *Strombina*-group. A pronounced suture runs through the capsule almost dividing it in two halves (Fig. 19). Although some capsules had preserved eggs, no larval shells were found in any of them. The protoconch (Fig. 20a, b) has a weakly sigmoid outer lip and 2.75 volutions at the time of metamorphosis.

Strombina (Recurvina) recurva **(G. B. Sowerby I, 1832)**
Strombina recurva (Fig. 21a, b) is the oldest living species of the *Strombina*-group known in the eastern Pacific, occurring as fossils in Late Miocene deposits of Ecuador (Jama formation) (Pilsbry & Olsson, 1941; Jung, 1989). This species is found alive usually at depths from a few meters to about 40 m deep, although it ranges from the intertidal zone to 240 m. Several shells with attached eggs were found in a lot of the Academy of Natural Sciences of Philadelphia that was dredged from a depth of 2-10 m on sand, east of Bahia Cocos, about 6 miles southwest of Puerto Culebra, Guanacaste, Costa Rica. The egg capsules were removed from the shell and fixed in 75% alcohol. Capsules are elliptical, with a basal membrane (Fig. 22). The capsules are smaller than those of *S. elegans*, but the eggs found in these capsules are the largest observed so far for any eastern Pacific species of the studied group (Table 1). Pre-hatching veliger shells with 1.75 whorls were found in several capsules (Fig. 23). The diameter of these intracapsular veliger shells is also the largest yet found in the group. The protoconch of *S. recurva* has 2.25 volutions (Fig. 24a, b), which indicates a period of further growth during the larval development.

Clavistrombina clavulus **(G. B. Sowerby I, 1834)**
The genus *Clavistrombina* is an eastern Pacific monotypic taxon. *C. clavulus* (Fig. 25) is usually found under rocks at low tides. This is not a common species, and usually is found alone. Several specimens were collected during the dry seasons of 1995-1997 in Playa Bique, Gulf of Panama (Fig. 1: stn. 3), and kept in an aerated aquarium at the STRI Naos marine laboratory. Here, animals usually hide under rocks brought from the collecting site. Contrary to other studied species of this group which were active all the time, *C. clavulus* emerged from its hiding place only when feeding. The short foot and relatively short siphon of this species is unique among all the species collected alive by us. In May-June 1997 ovoposition was observed in one of the aquaria where two specimens were kept. Several layers of egg capsules were deposited in two places of the walls of the aquarium. When we collected these capsules, more capsules were laid in the same spots. These capsules are quite distinct from all other species reported here. They are almost polygonal, with a large basal flange and an ellipsoid escape aperture in the center (Fig. 26). In some capsules, a circular depression can be seen around the escape aperture. Intracapsular development takes about 15 days. Veligers were seen actively feeding (and greenish filamentous microalgae were observed in the guts) after the 6th day of hatching. The hatching veliger shell has 1.5 whorls and a well developed siphonal canal with a reddish edge (Fig. 27). Veligers were alive for about two weeks after which they all died. The hatching of swimming and feeding veliger larvae, and the sinusigerous shape of the protoconch outer lip are evidence for planktotrophic development. Growth during this stage is also confirmed by the fact that the larval shell has 3 volutions at the time of metamorphosis (Fig. 28a, b).

Cosmioconcha modesta **(Powys, 1835)**
Live adult and juvenile *Cosmioconcha modesta* (Fig. 29a, b) were dredged during the dry season (January through April) of 1994-1996 from several localities in the Gulf of Panama (Venado, Bique, Farfan beaches), Taboga and Tabogilla islands, and Las Perlas archipelago (Fig. 1: stn. 1-3). Several adults were also collected in July 1995 from several localities in the Gulf of
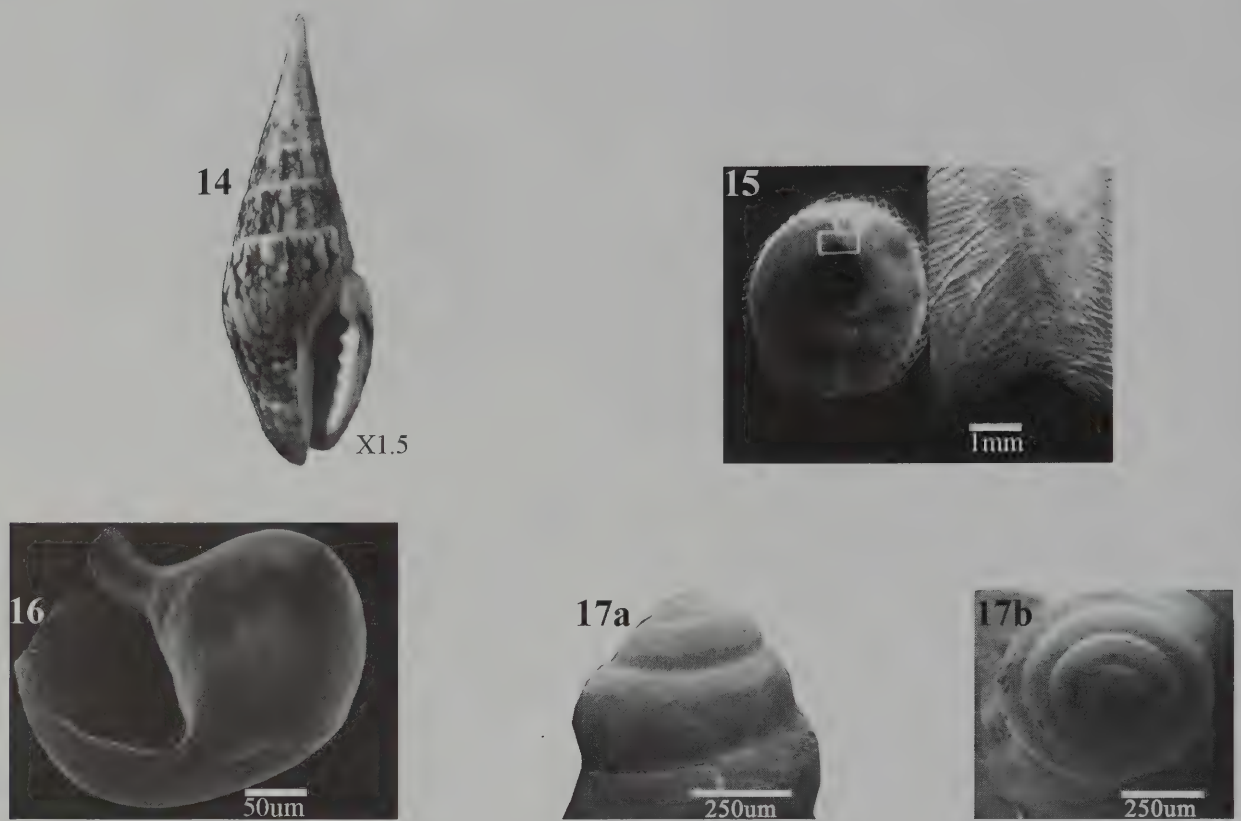
Figure 14-17. *Strombina (Spiralta) elegans* (B. G. Sowerby I, 1832) - Fig. 14: adult specimen (from JUNG, 1989 with permission); Fig. 15: SEM picture of the egg capsule (FMNH293340). The right side is a close up of the surface of the enclosed area in the left; Fig. 16: SEM picture of the veliger shell (FMNH293340); Fig. 17a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).
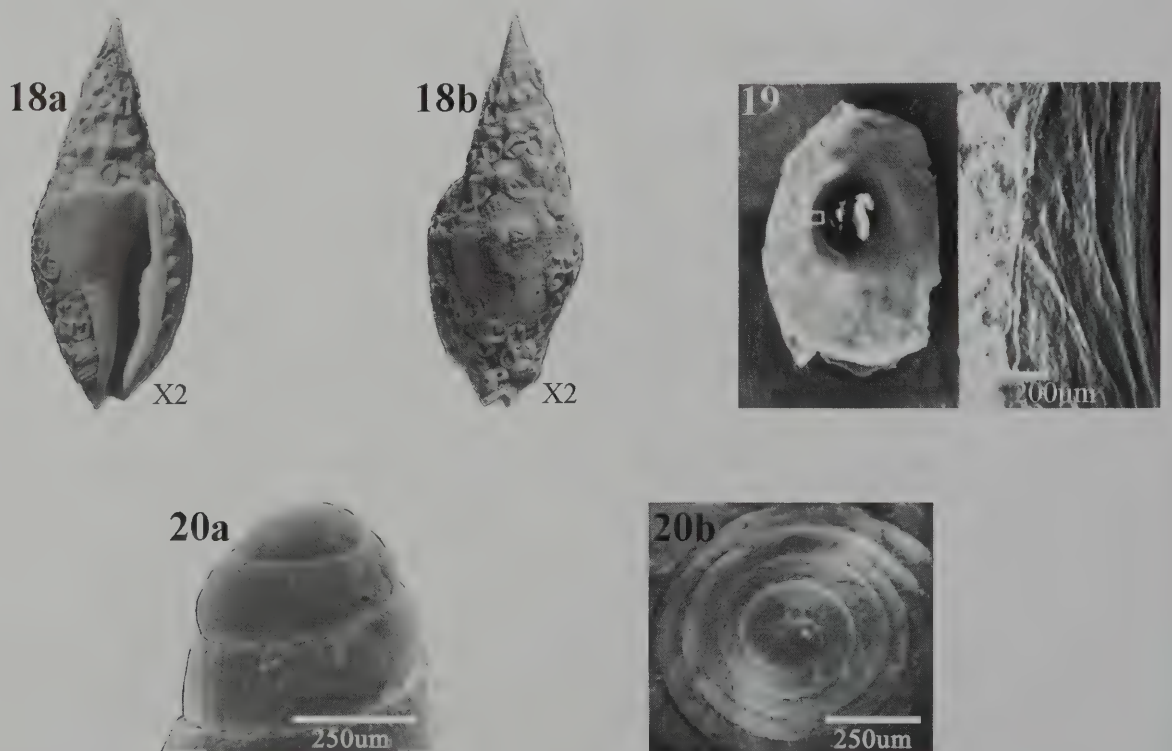
Figure 18-20. *Strombina (Strombina) lanceolata* (B. G. Sowerby I, 1832) - Fig. 18a-b: adult specimen (MHNG23078). Notice egg capsules attached to the shell. Fig. 19: SEM picture of the egg capsule. The right side is a close up of the surface of the enclosed area in the left; Fig. 20a-b: lateral and apical view of protoconch.

Chiriqui (Secas Is., Uva Is.) (Fig. 1: stn. 4) in muddy sands. As with other species reported here, the extremely long foot of *C. modesta* is well adapted for gliding on the surface of the mud. Egg masses collected in the field and laid in the laboratory were studied. Adults lay egg capsules on conspecific shells (Fig. 29b). An egg mass is usually composed of several layers of transparent, semispherical capsules with a well defined sutural ridge along the middle of the capsule and a basal flange (Fig. 30). A circular escape aperture is located in the center. Different embryonic stages were found in the same egg mass. Laboratory observations of oviposition show that a single egg mass is the result of several consecutive spawnings. Intracapsular development is similar to the other species reported here and occurs within 20 days. *Cosmioconcha modesta* hatches as a veliger with 1.75 whorls (Fig. 31) that actively swims and feeds (no special food was offered, but greenish filamentous microalgae were observed in the guts of the veligers). Veligers were kept alive for about two weeks after which they all died. The hatching of swimming and feeding veliger larvae, and the sinusigerous outer lip of the protoconch, are signs of planktotrophic development. At the time of metamorphosis, the larval shell has 2.5 whorls (Fig. 32a, b), which indicates that growth occurs during this free-swimming stage.

***Cosmioconcha parvula* (Dall, 1913)**
Live adults of *Cosmioconcha parvula* (Fig. 33) were dredged during July 1995 from a fine muddy bottom off Uva island in the Gulf of Chiriqui (Fig. 1: stn. 4), at a depth of 70 m. One of the animals had an egg mass on its shell composed of several dozen capsules. Capsules are small, roundish, with a median suture (Fig. 34). The elliptical escape aperture is located in the top center of the capsule and has a small basal membrane near the concave basal wall. Several capsules had small, yellowish eggs and others had embryos in different stages of development (Table 1). Intracapsular development was followed through hatching of the free-swimming planktonic veliger with 1.25 whorls. Further development was not followed because all the veligers died after 5 days. Although no signs of feeding were noticed during this period, the hatching of a competent swimming veliger larvae, the sinusigerous lip of the protoconch, and the fact that the larval shell has 2.5 volutions at metamorphosis (Fig. 35a, b) are evidences of planktotrophic development during which the larvae should feed and grow.

***Cosmioconcha redheri* (Hertlein & Strong, 1951)**
Several live specimens of *Cosmioconcha redheri* (Fig. 36) were dredged from 22-26 m near Uva island in the Gulf of Chiriqui in July 1995 (Fig. 1: stn. 4) in coarse sand with broken coral rubble. Thirteen egg capsules were found on the shell of a single individual. Capsules were in the pattern characteristic reported for the previous species. Capsules were round, with a wide flange near the concave basal wall (Table 1) and a unique pattern of circular lines (Fig. 37). The escape aperture is round and large relative to the size of the capsule (Table 1). There is no noticeable sutural ridge in the capsule. No larval shells were found in any of the collected material. The protoconch (Fig. 38a, b) has a weakly sigmoid outer lip and 3 volutions at the time of metamorphosis.

II - Caribbean Species

***Strombina (Strombina) pumilio* (Reeve, 1859)**
Strombina pumilio (Fig. 39) occurs commonly along the central and western coast of Venezuela in depths of 2-6 m, where it is usually buried in the top layer of coarse sand or among turtle and eel grass beds (Cipriani & Penchaszadeh, 1993). Animals can be located by their long and extremely mobile siphons that stick out of the sediment. Such behavior and mobility seems to be characteristic of most species of the *Strombina*-group observed so far. Adults lay translucent, domed-shaped egg capsules (Fig. 40) in masses placed on the dorsal side of conspecific shells. This species was shown to have direct development with fully developed juveniles crawling out of the capsule (Fig. 41) (Cipriani & Penchaszadeh, 1993). Protoconchs have 1.5 volutions (Fig. 42a, b) and a weakly sigmoid outer lip, which agrees with direct larval development.

***Strombina (Lirastrombina) francesae* J. Gibson-Smith, 1974**
Strombina francesae (Fig. 43) is apparently rare and restricted to Los Roques archipelago and surrounding areas along the central coast of Venezuela (Jung, 1989). This species is usually found in coarse sand where patches of algae drift are abundant (Cipriani & Penchaszadeh, 1993). Adult animals were found buried in the top 2 cm of sediment. Their shells were covered with several layers of transparent and hemispheric egg capsules (Fig. 44). Spawns, capsules and juvenile protoconchs (Fig. 45) were described by Cipriani & Penchaszadeh (1993). Observations on late embryos removed from egg capsules showed a well developed foot and cephalic tentacles, but no signs of a velum. The authors concluded that the species has direct development. This conclusion is supported by the weakly sigmoid shape of the protoconch outer lip and its maximum number of volutions (1.5) (Fig. 46a, b).

DISCUSSION AND CONCLUSIONS

Reproduction and development of the species of the *Strombina*-group reported here closely resembles that reported for most other columbellids, especially in the dome-shaped morphology of the egg capsule and the planktotrophic larval stage (Bandel, 1974, 1976; D'Asaro, 1970; Marcus & Marcus, 1962, 1964). The main difference is that most of the species reported here lay their eggs on the shells of conspecifics, even when other substrates are available. This maybe interpreted either as some kind of 'brood protection' or as an adaptation to a muddy habitat, or both.

Figure 47 shows characteristics of the protoconch for 54 species of the *Strombina*-group, both fossil and living which have been used to infer modes of development (modified from Jackson *et al.*, 1996). The larger, filled symbols indicate the eight species discussed in this paper whose larval development

Figure 21-24. *Strombina (Recurvina) recurva* (B. G. Sowerby I, 1832) - Fig. 21a-b: adult specimen (ANSP307933). Notice egg capsules attached to the shell; Fig. 22: SEM picture of the egg capsule. The right side is a close up of the surface of the enclosed area in the left: Fig. 23: SEM picture of the pre-hatching veliger shell; Fig. 24a-b: lateral and apical view of protoconch.
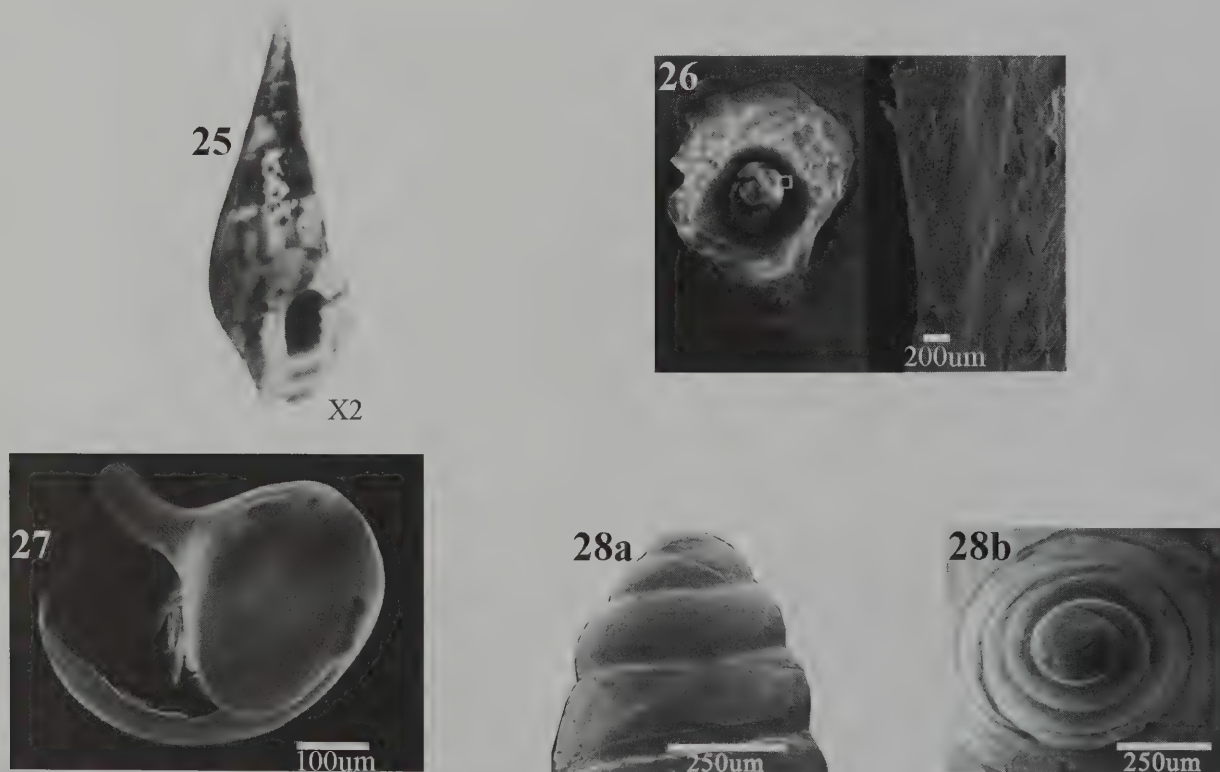
Figure 25-28. *Clavistrombina clavulus* (B. G. Sowerby I, 1834) - Fig. 25: adult specimen (from JUNG, 1989 with permission); Fig. 26: SEM picture of the egg capsule (FMNH293341). The right side is a close up of the surface of the enclosed area in the left; Fig. 27: SEM picture of the veliger shell (FMNH293341); Fig. 28a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).
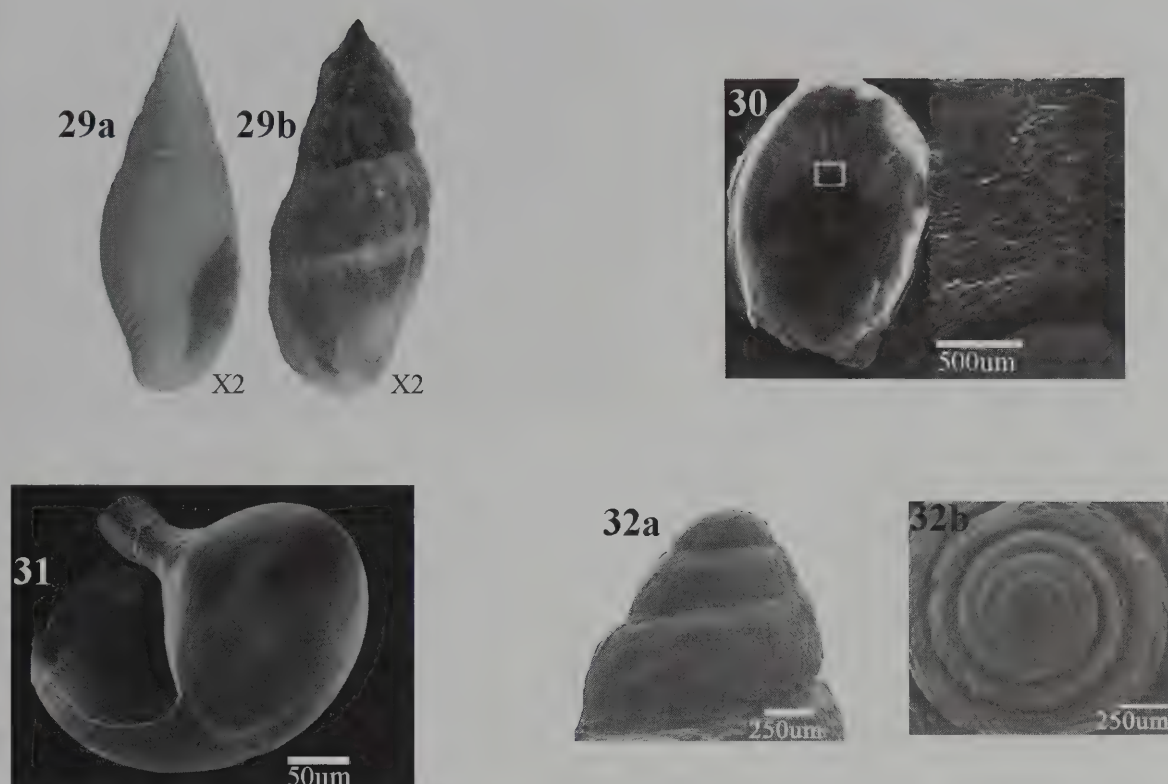
Figure 29-32. *Cosmioconcha modesta* (Powys, 1835) - Fig. 29a-b: adult specimen (FMNH293342). Notice egg capsules attached to the shell; Fig. 30: SEM picture of the egg capsule. The right side is a close up of the surface of the enclosed area in the left; Fig. 31: SEM picture of the veliger shell; Fig. 32a-b: lateral and apical view of protoconch.
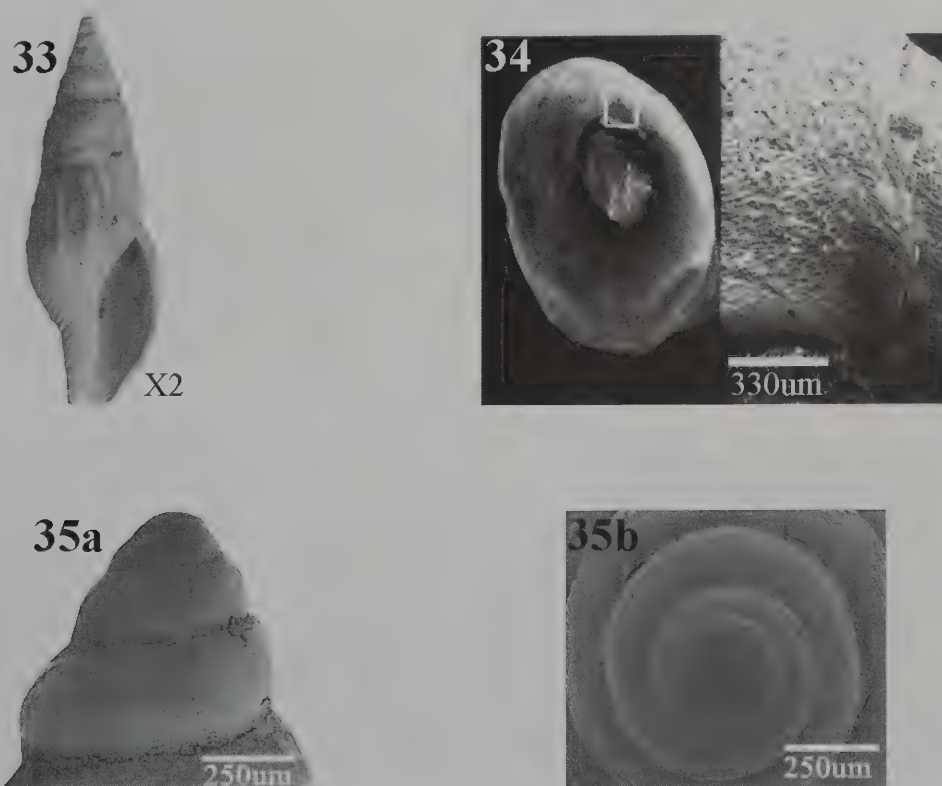
Figure 33-35. *Cosmioconcha parvula* (Dall, 1913) - Fig. 33: adult specimen (FMNH280885); Fig. 34: SEM picture of the egg capsule. The right side is a close up of the surface of the enclosed area in the left; Fig. 35a-b: lateral and apical view of protoconch.
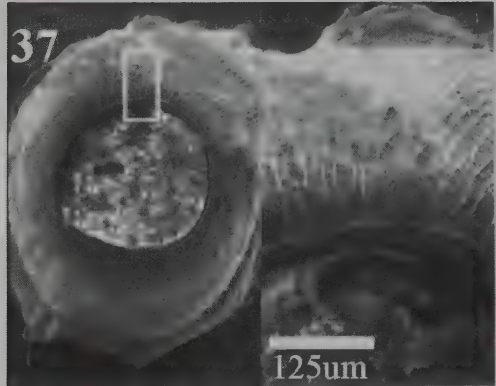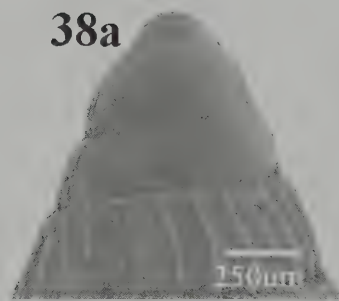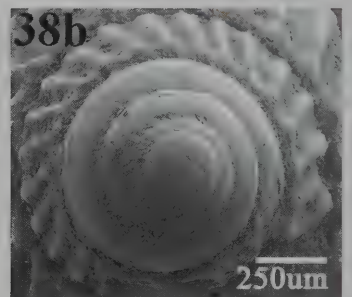
Figure 36-38. *Cosmioconcha redheri* (Hertlein&Strong, 1951) - Fig 36: adult specimen (FMNH280886); Fig. 37: SEM picture of the egg capsule. The right side is a close up of the surface of the enclosed area in the left; Fig. 38a-b: lateral and apical view of protoconch.
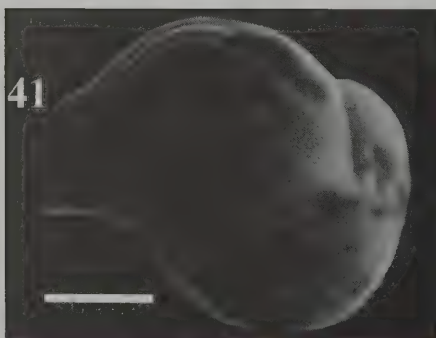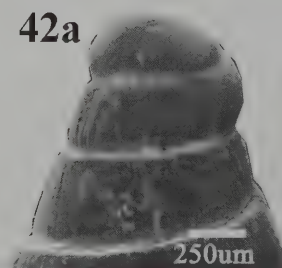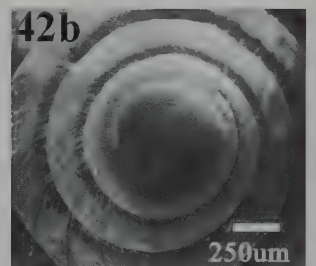
Figure 39-42. *Strombina (Strombina) pumilio* (Reeve, 1859) - Fig. 39: adult specimen (from JUNG, 1989 with permission); Fig. 40: SEM picture of the egg capsule, scale bar 5mm (fom CIPRIANI & PENCHASZADEH, 1993 with permission); Fig. 41: SEM picture of the veliger shell, scale bar 0.25mm (from CIPRIANI & PENCHASZADEH, 1993 with permission); Fig. 42a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).

has been observed independently, rather than derived from measurements and observations of the larval shell. There is a good agreement between inferred and observed patterns of development for planktotrophic and direct developers, which lends strong support to the continued use of morphological data to infer patterns of development for fossil taxa (SCHELTEMA, 1977, 1978; JABLONSKI & LUTZ, 1979, 1980, 1983; HANSEN, 1980, 1982; JABLONSKI, 1982, 1986). However, more direct biological observations of life histories are necessary to refine the limits and the criteria to be used to infer types of larval development for different taxa. In the case of the *Strombina*-group, the best criteria to evaluate development types seem to be the number of protoconch volutions. Although the maximum diameter of the protoconch has been used to infer types of development of other gastropods, it contributes less information for species of the studied group.

There was no clear relation between the number and size of eggs or capsules and the observed mode of larval development because all these parameters vary enormously among planktotrophic species as can be seen in Table 2. Possibly, the number of eggs per capsule or the number of eggs per capsule normalized to capsule size may prove useful with observations of more species, at least for the distinction of species with direct development from the rest (Fig. 48).

On the other hand, our data is not enough to deduce any definite pattern concerning lecitotrophic species. Of all the species reported here, *Strombina recurva* could be classified as a possible candidate for this type of development. The fact that the intracapsular veligers found had 1.75 volutions and the protoconch has 2.25 indicates a further period of growth. The question is where does this growth takes place, inside the capsule or in the plankton. The sinusigerous shape of the protoconch outer lip leads us to think that some of this growth could take place during a planktotrophic stage. Besides, the protoconchs of most of the known direct developers have fewer whorls. More data on this and other possible lecitotrophs is needed in order to be able to deduce this type of development with more confidence.

Finally, the new observations reported here allow considerable refinement of the contrasting evolution of larval developmental modes in the eastern Pacific and Caribbean. Figure 49 (modified from JACKSON *ET AL.*, 1996) shows the frequency of the three developmental modes for 73 species of the *Strombina*-group since the Early Miocene and the possible relation between developmental mode and geographic range. The proportion of species with direct development increased progressively in the Caribbean even before the mass extinction at the end of the Pliocene as reported previously (JACKSON *ET AL.*, 1996).
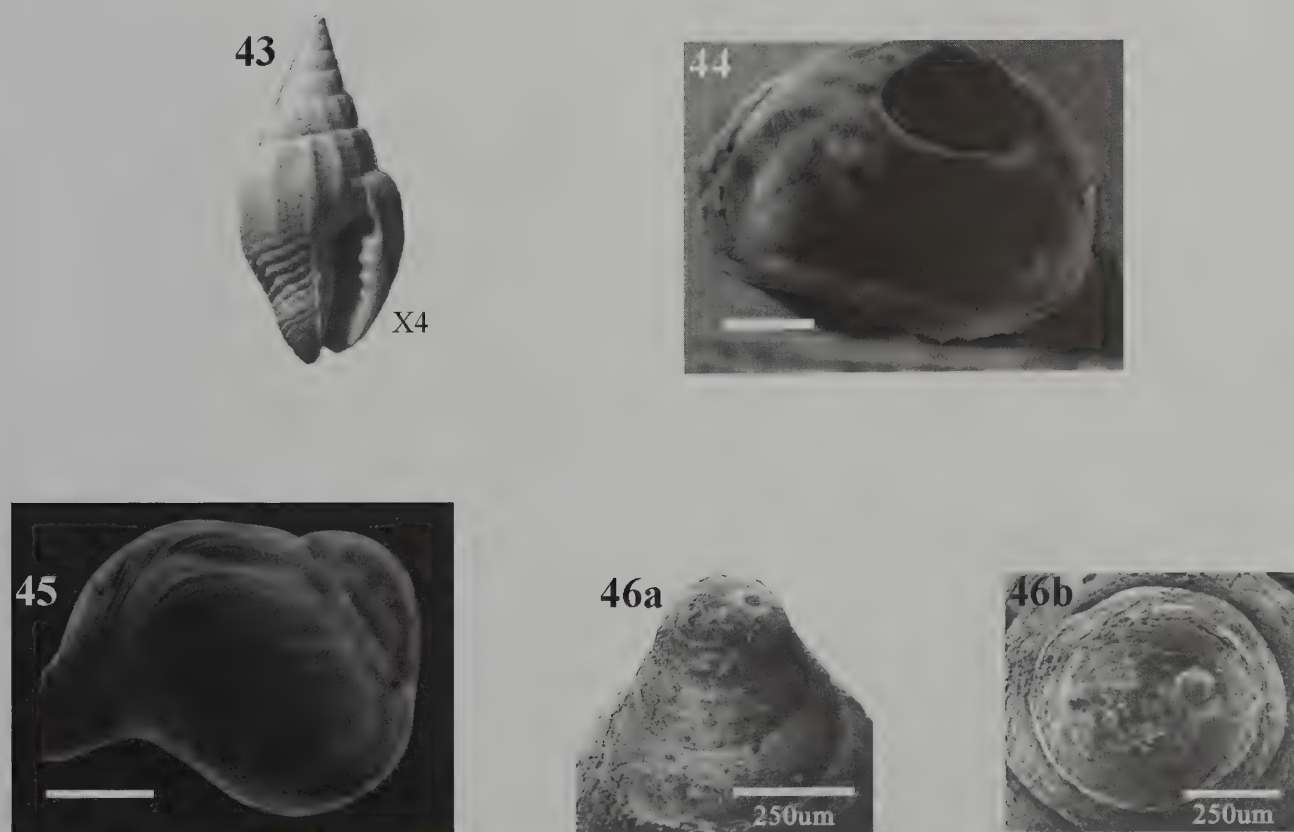
Figure 43-46. *Strombina (Lirastrombina) francesae* J. Gibson-Smith, 1974 - Fig. 43: adult specimen (from JUNG, 1989 with permission); Fig 44: SEM picture of the egg capsule, scale bar 0.25mm (from CIPRIANI & PENCHASZADEH, 1993 with permission); Fig. 45: SEM picture of the veliger shell, scale bar 0.25mm (from CIPRIANI & PENCHASZADEH, 1993 with permission); Fig. 46a-b: lateral and apical view of protoconch (from JUNG, 1989 with permission).

However, lecithotrophic development was less important in the eastern Pacific than surmised previously, an observation that is in better agreement with data from echinoderms (LESSIOS, 1990) and corals (RICHMOND & HUNTER, 1990).

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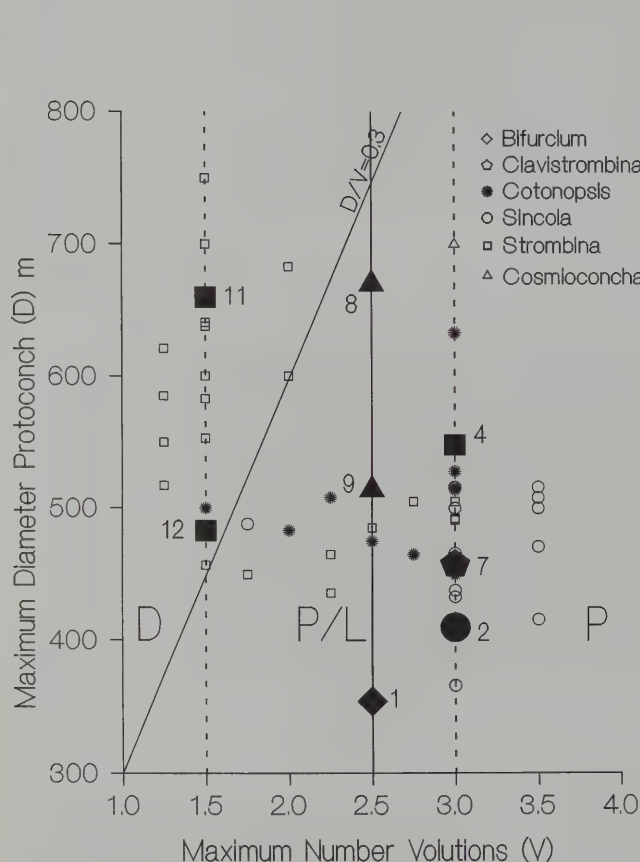
Figure 47. Relation between the maximum diameter (D) and maximum number of volutions (V) of the larval shell (protoconch) for 54 species of the *Strombina*-group, fossil and living, used to infer modes of development (modified from JACKSON *ET AL.* 1996). The larger, filled symbols indicate the eight species discussed in this paper whose larval development has been observed independently, rather than derived from measurements and observations of the larval shell. The vertical dashed lines separate the graph into three areas of inferred development based on the maximum number of protoconch volutions: D - direct development ($V < 1.5$); P/L - planktotrophic or lecithotrophic development ($1.5 < V < 3.0$); P - planktotrophic development ($V >= 3.0$). The values of D and V for $D/V = 0.30$ are designated by the solid oblique line. The vertical solid line constrains the values of V for lecithotrophic species.
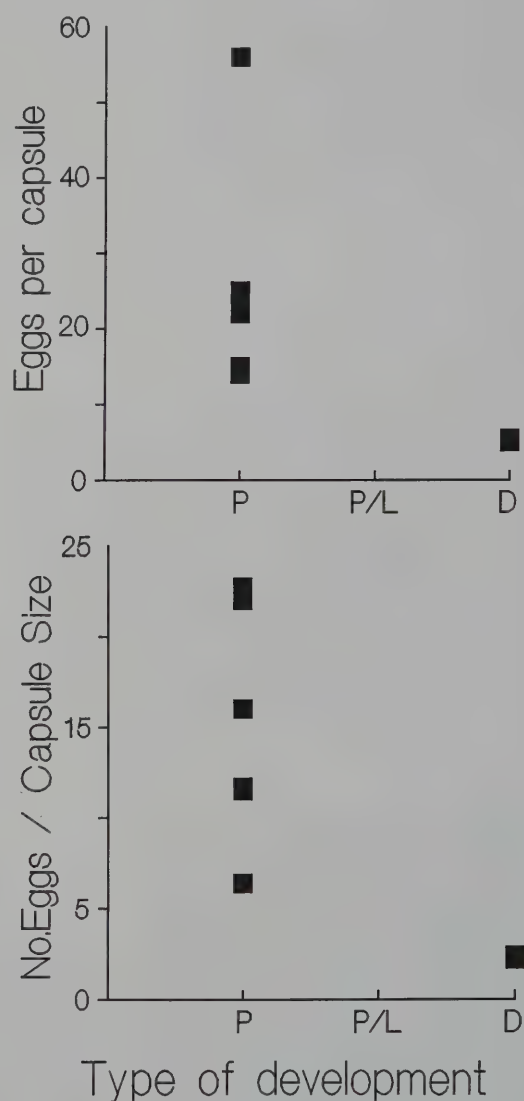
Figure 48. Relation between the number of eggs per capsule and the number of eggs per capsule normalized to capsule size and the type of development for eight species of the *Strombina*-group. These are the same species represented by filled large symbols in figure 47.

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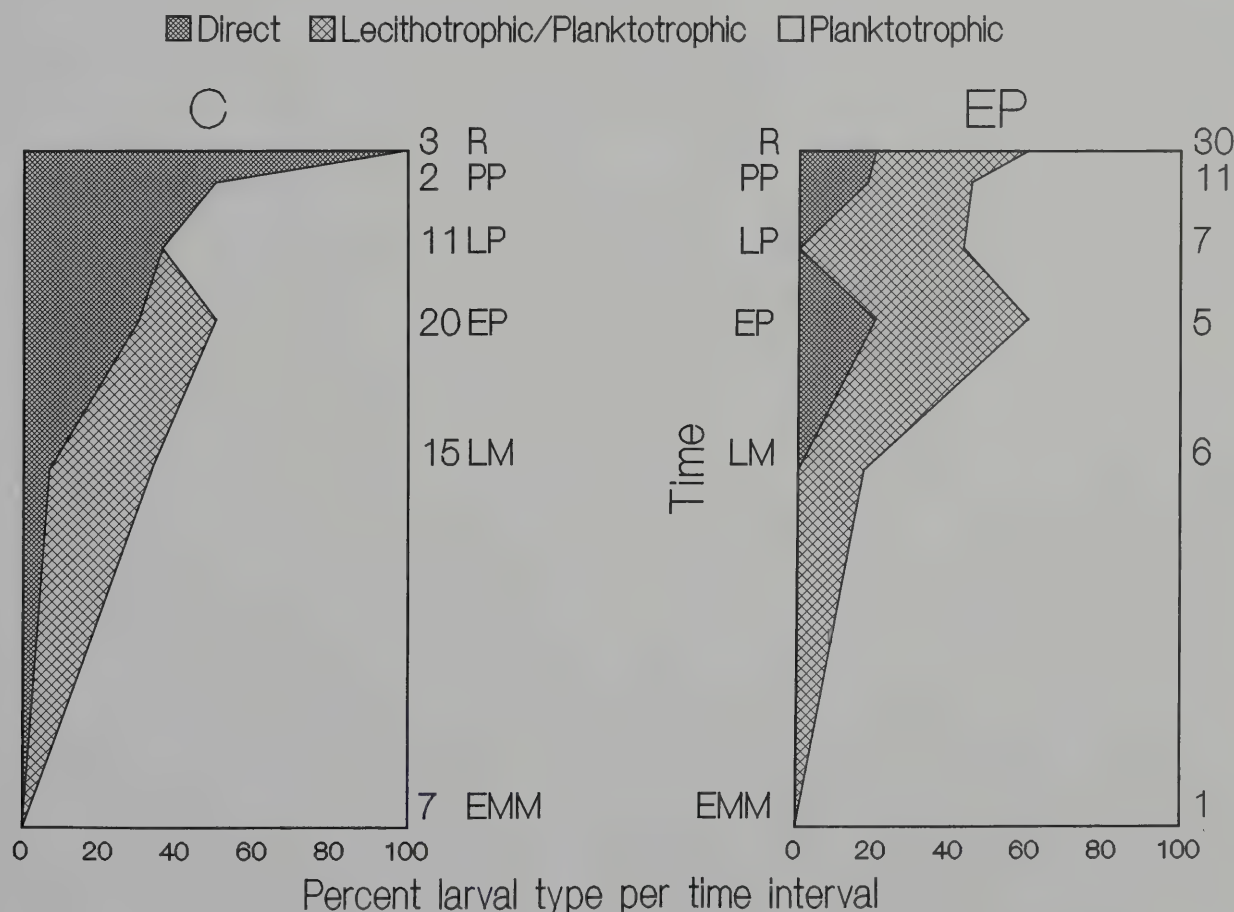
Figure 49. Temporal changes in the relative numbers of species of the *Strombina*-group inferred to have planktotrophic (P), either planktotrophic or lecithotrophic (P/L), and direct (D) development. A total of 73 species (fossil and Recent), from the eastern Pacific (EP) and the Caribbean (C) for which values for the maximum number of volutions and the maximum diameter of the protoconch could be evaluated, were analyzed. Numbers to the right of the boxes represent the number of species per time interval evaluated for each ocean. Time intervals considered are: Recent (R), Plio-Pleistocene (PP), Late Pliocene (LP), Early Pliocene (EP), Late Miocene (LM), Early-Middle Miocene (EMM).

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Systematics and phylogeny of the genus *Trophon* Montfort, 1810 (Gastropoda: Muricidae) from Patagonia and Antarctica: morphological patterns

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KEY WORDS: *Trophon,* Systematics, biogeography, comparative morphology.

ABSTRACT The systematics and phylogeny of species commonly included under the genus *Trophon* from Patagonia and Antarctica were studied. A bibliographic survey established the existence of over 100 specific names that have been proposed for living and fossil representatives of the genus *Trophon* from the study area. The following questions were addressed: How many valid species belonging to the genus *Trophon* live nowadays in Patagonia and Antarctica? Which were their real geographic ranges? Which valid genera of Trophoninae are represented in the study area? Is the subfamily Trophoninae a monophyletic group?

The specimens studied were drawn from the collections of the Museums of, La Plata and Buenos Aires, (Argentina), and the National Museum of Natural History, Smithsonian Institution, (USA). Material collected along the entire Patagonian coast was also included. Approximately 1,000 specimens, in more than 600 lots, were compared with the holotypes housed in several European and American institutions. Radular, anatomical and shell characters were used to redefine each species. Preliminary results of this analysis yielded 33 valid species that have been previously described and at least five new species. Two basic different morphological patterns are proposed using gross anatomy, accesory salivary glands and radular features. The morphological arrangement suggested that the Patagonian species group and the Antarctic species group heretofore considered to be in the same genus, are probably polyphyletic. The Patagonian group showed close relationships among its representatives.The species belonging to the Antarctic group are less known and further studies will probably show that this group includes representatives of different clades.

RIASSUNTO Sono qui studiate la sistematica e la filogenesi delle specie comunemente incluse nel genere *Trophon* dalla Patagonia e dall'Antartide. Una revisione dei dati bibliografici dimostra l'esistenza di oltre 100 nomi specifici proposti per rappresentanti fossili e viventi del genere *Trophon* dall'area di studio. Le seguenti questioni vengono esaminate: quante specie valide appartenenti al genere *Trophon* vivono attualmente in Patagonia e in Antartide? Qual'è la loro reale distribuzione geografica? Quali generi validi di Trophoninae sono rappresentati nell'area di studio? La sottofamiglia Trophoninae è un gruppo monofiletico?

Gli esemplari studiati originano dale collezioni dei musei di La Plata and Buenos Aires, (Argentina), e dal National Museum of Natural History, Smithsonian Institution, (USA). Materiale raccolto lungo l'intera costa patagonica è stato inoltre incluso. Circa 1000 esemplari, in oltre 600 lotti, sono stati comparati con gli olotipi conservati in varie istituzioni europee ed americane. Caratteri anatomici, radulari e conchiliari sono stati impiegati nella ridefinizione delle specie. I risultati preliminari di tale analisi hanno prodotto evidenze per 33 specie valide già descritte, e per almeno cinque specie non descritte. Due pattern morfologici distinti sono proposti sulla base dei dati anatomici generali, ghiandole salivari accessorie e caratteristiche radulari. La classificazione morfologica proposta suggerisce che il gruppo di specie patagoniche e quello di specie antartiche, finora considerati appartenenti al medesimo genere, siano probabilmente polifiletici. Le specie patagoniche hanno mostrato un considerevole livello di affinità tra di loro. Le specie appartenenti al gruppo antartico sono meno conosciute, e ulteriori ed approfonditi studi sono necessari, e probabilmente mostreranno che questo gruppo include rappresentanti di diversi cladi.

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INTRODUCTION

The genus *Trophon* was originally proposed for *Buccinum geversianus* Pallas, 1774 an extremely variable species whose real range includes both coasts of southern South America. Shell characters in this species are highly variable, and together with its widespread geographical range have brought about an extraordinary proliferation of names. VOKES (1991) included 14 different specific names which had been used only for *Trophon geversianus*, most of them synonymous. Vokes herself restricted the genus to *Trophon* s.s., with two species, also drawing attention to the presence in the area of the genera *Xymenopsis* Powell, 1951 (with two species) and *Fuegotrophon* Powell, 1951 (with one species). The 26 different names given to these three species has hindered the accurate interpretarion of their relationships. Most work on systematics of *Trophon* has been based on shell morphology, while anatomical data have been so far badly misappraised.

As a fossil *Trophon* can be identified in Patagonian rocks of an age as early as at least the early Eocene. IHERING (1907) mentioned nearly 15 species included in *Trophon s. l.*. However, modern revisions could possibly modify this arrangement substantially.

A bibliographic survey established the existence of over 100 specific names that have been proposed for living and fossil representatives of the genus *Trophon* from the study area (see apendix 1).

The last good account of the genus *Trophon* from Antarctica was accomplished by POWELL in 1951 and 1958. He described one genus, two subgenera and four new species. Powell included for the first time, illustrations of the radula and protoconch of several species, despite of previous THIELE'S (1904) illustration of the radula of *T. albolabratus* Smith. These illustrations clearly point out several different radular morphologies. However, the quality of the drawings never allowed an accurate comparison with the other representatives.

Recent attempts (NUMANAMI, 1996; HOUART, 1997; 1998) to include the antarctic species of this genus worked basically by default for all the fusiform lamellose shells with tricuspid rachidian radulae teeth regardless of their real affinities. Comparative studies on the soft anatomy and radulae on Antarctic representatives of the subfamily Trophoninae are wanted.

In this paper the following questions are addressed: How many valid species belonging to the genus *Trophon* live nowadays in Patagonia and Antarctica? Which are their real geographic ranges? Which valid genera of Trophoninae are represented in the study area? Is the subfamily Trophoninae a monophyletic group?

MATERIAL AND METHODS

The specimens studied were drawn from the collections of the Museums of La Plata (MLP) and Argentino de Ciencias Naturales (MACN), Buenos Aires, (Argentina), and the National Museum of Natural History, Smithsonian Institution (USNM), Washington, D.C. (USA). Material collected along the entire Patagonian coast was also included. Approximately 1,000 specimens, in more than 600 lots, were compared with the holotypes, paratypes and syntypes housed in several European and American institutions.

Most of the material are from the United States Antarctic Program (USAP) housed at USNM. This material belongs basically to the expeditions of three ships: RV/ Hero, RV/ Eltanin and RV/Siedlecki. Additional specimens are from several Antarctic expeditions of the Argentine Republic, deposited at the MACN. Finally, several lots were studied at the Zoological Institute and Museum, Hamburg (ZMH) and Senckenberg Museum, Frankfurt (SMF), most of them belonging to the expeditions of the ships RV/Walther Herwig and RV/Polarstern.

The animals, all preserved in alcohol, were dissected to extract the radulae and to illustrate the gross anatomy of the anterior parts of the digestive system including salivary glands, accessory salivary glands and stomach when it was available. In addition, the morphology of the penes and female external organs are presented, some of them processed with critical point dry.

The radulae were prepared according to the method described by SOLEM (1972) and observed under the scanning electron microscope (SEM). All pictures were digitalized with a digital scanning camera and processed with the software Adobe Photoshop v. 6.01.

RESULTS

The study of the material allows the following systematic arrangement:

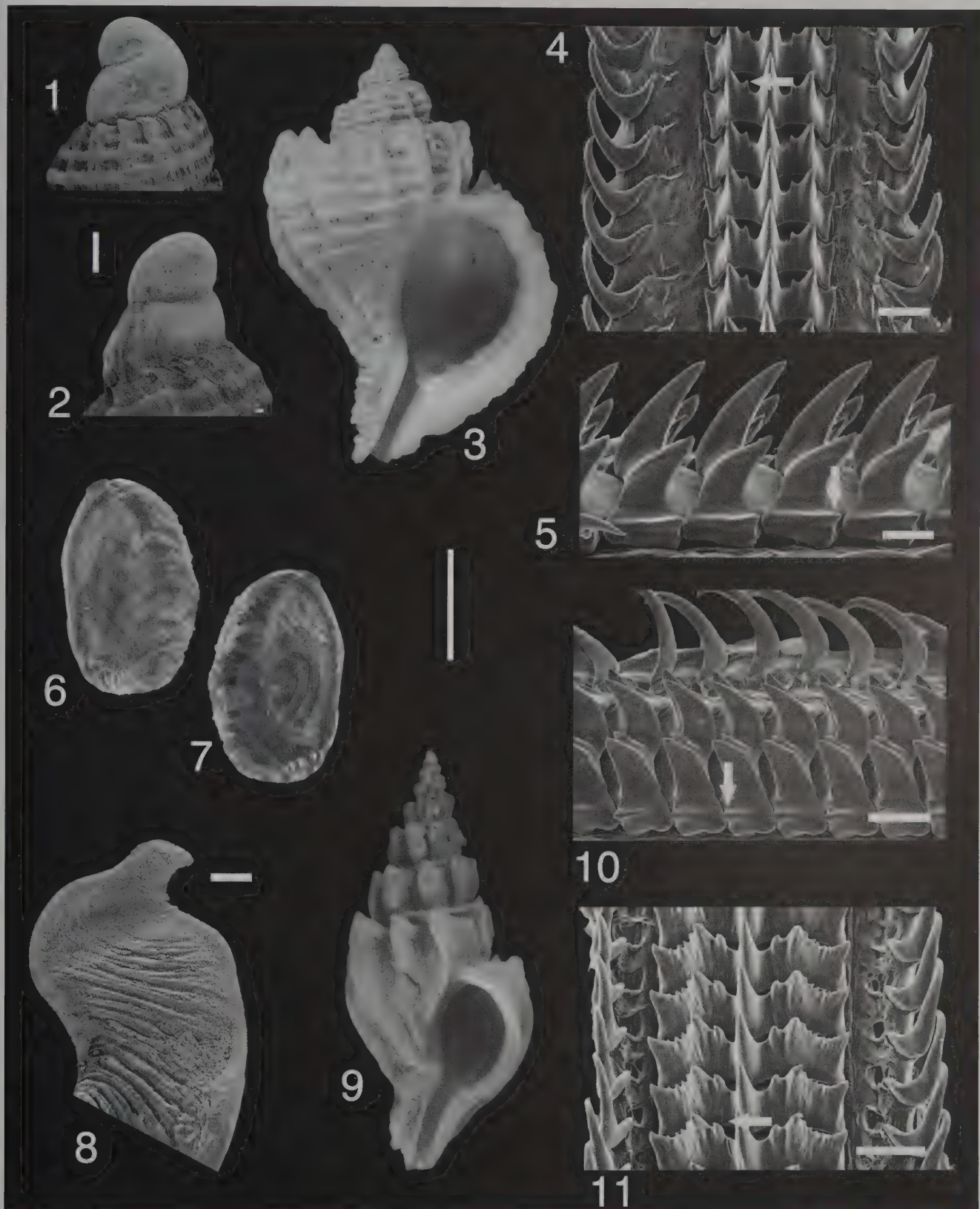
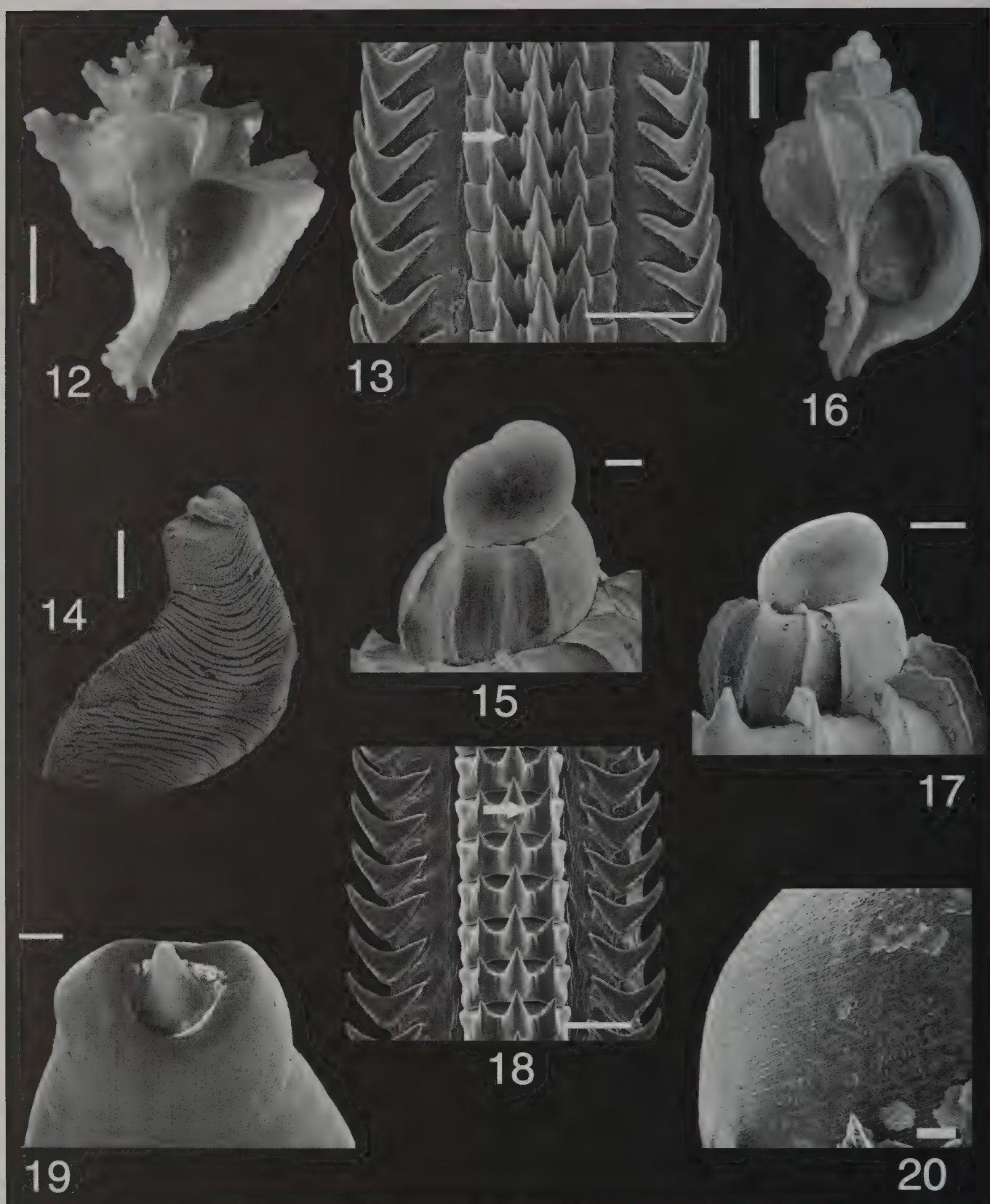
Trophon

T. geversianus (Pallas, 1774)
T. plicatus (Lightfoot, 1786)
T. pelseneeri (Smith, 1915)
T. bahamondei McLean & Andrade, 1982
T. iarae Houart, 1998
T. acanthodes Watson, 1882

"T." malvinarum Strebel, 1908
"T." ohlini Strebel, 1904
"Murex" clenchi Carcelles, 1953

Polar *"Trophon"*
T. mawsoni Powell, 1957
T. leptocharteres Oliver & Picken, 1984
T. macquariensis Powell, 1957
T. nucelliformis Oliver & Picken, 1984
T. cribellum Strebel, 1908
T. cuspidarioides Powell, 1951
T. septus Watson, 1882
T. scolopax Watson, 1882
T. scotianus Powell, 1951
T. coulmanensis Smith, 1907
T. shackletoni Hedley, 1911
T. paucilamellatus Powell, 1951
T. declinans Watson, 1882
T. longstaffi Smith, 1907
T. minutus Melvill & Standen, 1907

Table I. Geographic distribution of Trophon species from Southern South America and Antarctica. O=South Orkney; K= Kerguelen; G= South Georgia; M=Macquarie Is.

Magellanic species	Insular species	Circumantarctic
T. geversianus (Pallas)	*Trophon cribellum* Strebel (G)	*Trophon coulmanensis* Smith
T. plicatus (Lightfoot)	*T. albolabratus* Smith (K)	*T. drygalskii* Thiele
T. pelseneeri (Smith)	*T. brevispira* Martens (G)	*T. echinolamellatus* Powell
T. bahamondei McLean & Andrade	*T. distantelamellatus* Strebel (G)	*T. minutus* Melvill & Standen
T. iarae Houart	*T. macquariensis* Strebel (G)	*T. enderbyensis* Powell
T. malvinarum Strebel	*T. mawsoni* Powell (M)	*T. minutus* Melvill & Standen
T. acanthodes Watson	*T. scolopax* Watson (K)	*T. longstaffi* Smith
	T. septus Watson (K)	*T. scotianus* Powell
"T". ohlini Strebel	*T. cuspidarioides* Powell (G)	*T. shackletoni* Hedley
"Murex" clenchi Carcelles	*T. eversoni* Houart (K?)	*T. nucelliformis* Oliver & Picken
	T. leptocharteres Oliver & Picken (O)	*T. declinans* Watson
		T. paucilamellatus Powell

Figures 1-11. Patagonian *Trophon* 1-7. *Trophon geversianus* (Pallas, 1774), MLP 27201, Puerto Golondrina, Ushuaia, Tierra del Fuego. 1-2. Two side views of the protoconch, scale bar = 500 µm. 3. Shell apertural view, Scale bar = 1 cm. 4. Dorsal view of radular ribbon, arrow heads internal denticle of lateral cusp of rachidian tooth, scale bar = 50 µm. 5. Lateral view of rachidian teeth, arrow heads single marginal cusp, scale bar = 30 µm. 6-7. Operculum, external (6) and internal (7) views, scale bar = same as 3. 8-11. *Trophon plicatus* (Lightfoot, 1786). 8. Penis, critical-point dried, scale bar = 400 µm. 9. Shell apertural view, scale same as in 3. 10. Lateral view of rachidian teeth, arrow heads single marginal cusp scale bar = 50 µm. 11. Dorsal view of radular ribbon, arrow heads internal denticle of lateral cusp of rachidian teeth scale bar = 50 µm.

Figures 12-20. Antarctic *Trophon* 12-15. *Trophon paucilamellatus* Powell, 1951, USNM 897576,. 12. Shell apertural view, scale bar = 1 cm. 13. Dorsal view of radular ribbon, arrow heads internal denticle rising from the base of rachidian tooth, scale bar = 100 µm. 14. Penis, critical-point dried, scale bar = 500 µm. 15. Protoconch, scale bar = 200 µm. 16-20. *Trophon scotianus* Powell, 1951. 16. Shell apertural view, scale bar = 1 cm. 17. Protoconch, scale bar = 200 µm. 18. Dorsal view of radular ribbon, arrow heads obsolete intermediate denticle between lateral and central cusp of rachidian teeth, scale bar = 200 µm. 19. Tip of the penis, critical-point dried, scale bar = 400 µm. 20. Protoconch, detail showing the spiral ornamentation, scale bar = 60 µm.

T. drygalskii Thiele, 1912
T. brevispira Martens, 1885
T. distantelamellatus Strebel, 1908
T. enderbyensis Powell, 1958
T. echinolamellatus Powell, 1951
T. eversoni Houart, 1997
T. albolabratus Smith, 1875
T. mucrone Houart, 1991
T. veronicae Pastorino, 1999

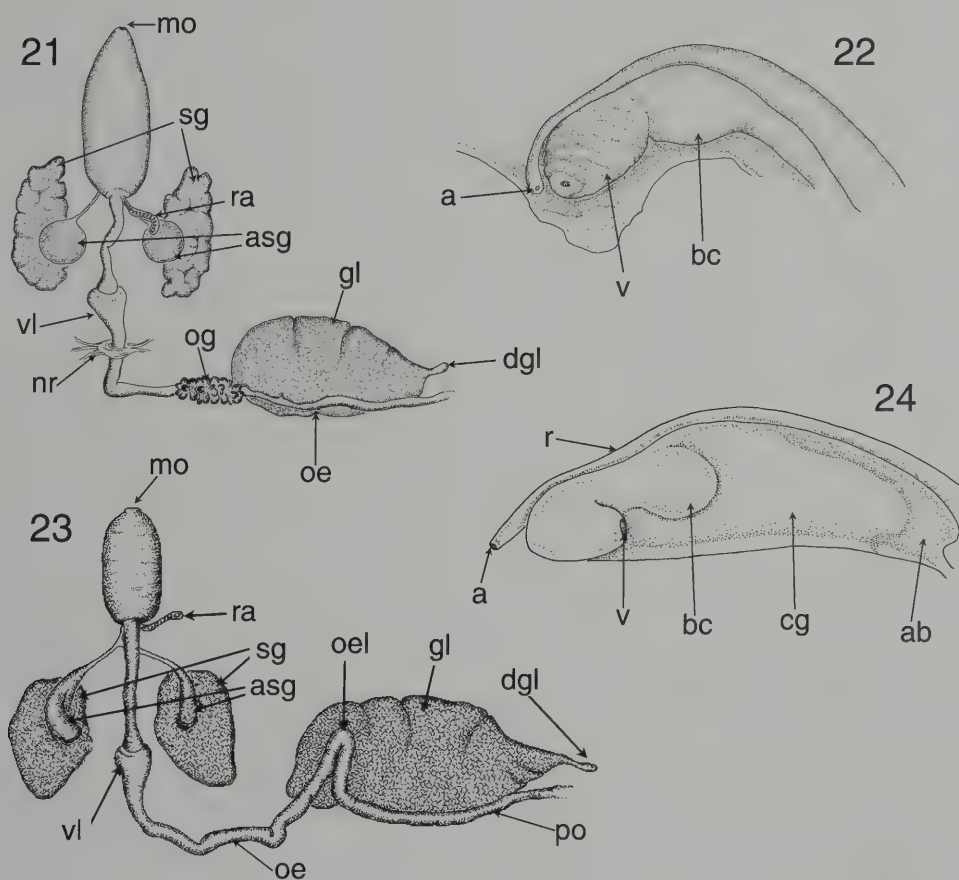
Coronium
C. coronatum (Penna-Neme & Leme, 1978)
C. elegans Simone, 1996
C. oblongum Simone, 1996

Fuegotrophon
F. pallidus (Broderip in Broderip & Sowerby, 1833)
F. amettei (Carcelles, 1946)

Xymenopsis
X. corrugata (Reeve, 1848)
X. muriciformis (King & Broderip, 1832)
X. subnodosa (Gray, 1839)
X. buccinea (Lamarck, 1816)

Tromina
T. dispectata Dell, 1990

Boreotrophon
B. verrillii (Bush, 1893)
B. aculeata (Watson, 1882)

This arrangement includes all living species considered valid up to now described in the family Trophoninae from South America and Antarctica. There are several remarks to take into account. *Murex clenchi* is only known by three specimens without soft parts including holotype and paratype. Protoconch and

Figures 21-24. Diagrammatic schemes of the alimentary and paleal female reproductive systems. 21. *Trophon paucilamellatus* Powell, 1951. anterior portion of the alimentary system. 22. *T. equinolamellatus* paleal reproductive female organs. 23. *Trophon plicatus* (Lightfoot, 1786), anterior portion of the alimentary system. 24. anterior portion of the alimentary system.
a = anus; ag = albumen gland; ap = anal papilla; asg = accessory salivary gland; bc = bursa copulatrix; cg = capsule gland; dgl = ampulla of gland of Leiblein; gl = gland of Leiblein; mo = mouth; nr = nerve ring; oe = oesophagus; og= oesophageal glands ("Framboisse glands"); oel: oesophageal loop; po= posterior oesophagus; r = rectum; ra = radula; sg = salivary gland; v = vagina; vl = valve of Leiblein.

ultrastructure of the shell suggests that it belongs to the genus *Trophon*, however, the radula will have the last word about it. Something similar occurs with *T. malvinarum* which is only known by the holotype and a few dry specimens.
Trophon ohlini was originally described under this genus by Strebel. However, the radula and the protoconch are actually very different from all the other species. It will probably belong to a different genus.
The analysis of the radulae and gross anatomy of most of the species up to now described suggests the presence of two basic morphological types. I selected two representatives species as an example of both morphological arrangements: *Trophon geversianus* (Figures 23 and 24) from the Magellanic Malacological Province and *Trophon paucilamellatus* from Antarctic and circum-antarctic waters (Figures 21 and 22).

DISCUSSION

Radular remarks

Bouchet and Warén (1985) in the first attempt to study, with modern criteria, the species included in the genus *Trophon* from the Northern Atlantic considered no heavy taxonomic differences among the species from Northern and Southern Hemisphere. However, a close review of Patagonian and Antarctic species demonstrate that both groups are really different and probably belongs to several distinct genera. Bouchet and Warén (1985) mentioned the opercular features proposed by Radwin & D'Attilio (1976) and Vokes (1976) as insufficient to separate both groups. This is probably true for the Patagonian and boreal species which show a very similar pattern of opercular morphology. They also mentioned the common features of the radulae of Northern and Southern *Trophon*. This is partially accurate. Most of the Patagonian species that belong in this genus have several common characteristics that are different from the Antarctic and Boreal ones.
Radulae of Patagonian *Trophon* have always the intermediate denticle in the inner upper part of the lateral cusps of the rachidian teeth. The radulae of Boreal species showed by Bouchet and Warén (1985), as far as it can be seen in their illustrations, have different positions of these intermediate denticles, but never as in *T. geversianus* despite what they suggested.

All the antarctic species so far described as *Trophon* never present this radular arrangement. The typical radula of "*Trophon*" from Antarctica have a tricuspid rachidian teeth with the intermediate denticles completely separate from the inner margin of lateral cusps or obsolete but always rising from the base (see Figures 13, 18).

The antarctic and boreal species of *Trophon* have the inner denticle between central and lateral cusp of the rachidian teeth always free, attached to the base of the teeth. Most of the radulae of Northeast Atlantic species of *Trophon* (as far as I can see in Bouchet and Warén's revision (1985) have a broad attachment of the marginal teeth.

Based on radular features the whole *Trophon* group from Patagonia is very homogeneous. All the species included in this group have the following common radular features (Figures 4-5, 10-11):

1-intermediate denticle attached to the upper height of the internal edge of the lateral cusp of the rachidian teeth;
2-single marginal denticle in the external edge of the base of the rachidian teeth;
3-the attachment area of the marginal teeth are always (no exception) narrow,
thin and the free part of the same thickness,
4-the central cusp of the rachidian is always thin and larger than the laterals.

Conchological remarks

Conchological features are so variable that I considered them secondary. However, the protoconch is actually very different and permits the division in at least both two clearly defined groups. Most of the Patagonian representatives have a slightly asymmetrical protoconch in relation with the axis (Figures 1-2) which is mostly perpendicular to the shell axis in the Antarctic group. There is no ornamentation in Patagonian *Trophon,* whereas most of the boreal species have a delicate pattern of irregular cords. The antarctic species have in general no ornamentation in the protoconch either with only three exceptions: *T. scotianus* Powell, 195, *T. drygalskii* Thiele, 1912 and *T. paucilamellatus* Powell, 1951 which have apparently the same pattern that it could be observed in North Atlantic species (see Figures 17, 20). No protoconch of the genus *Trophon* from Patagonia has spiral ornamentation, as is almost the rule in the boreal Atlantic species (see figs. 289, 290,295, 296, 299, 300, 305 in Bouchet & Warén, 1985).

Anatomical remarks

There are several gross anatomical features that characterize the group living in the South American coast. Where known, the accessory salivary glands are usually tubular ("kidney-shape"). As a rule the oesophagus produces a typical oesophageal loop before the valve of Leiblein and posteriorly runs appresed to the left side of this gland. The oesophageal glands ("Framboisse" glands) in the mid-oesophagus are inconspicuous, not externally visible. Finally, penes are always dosoventrally flattened, with a large papilla and a simple *vas deferens* closed by the overlapping sides of the penis which could be open in some species (*e.g. T. geversianus*, Kool, 1993).
On the other side, most of the Antarctic species have spherical accessory salivary glands usually embedded in the salivary glands. The oesophagus runs immediately towards and under the gland of Leiblein, but previously a very developed (visible to naked eye) oesophageal glands are present.
Penes are in fact very different in most of both groups of species. However, the Patagonian group presents papillae, large and long, or short and small but never conical and rising from the middle or the ring-like penis tip as it is usual in several of the Antarctic species (Figures 8, 14,19).
The palial oviduct arranged as in *Trophon geversianus* is the generalized morphology for the Patagonian species (Harasewych, 1984). As an example of the antarctic representatives, *Trophon scotianus* has a broad pallial oviduct and the bursa copulatrix oval, globose with several layers of tissue before leads to the vaginal opening.

Geographical remarks

Table I shows living *Trophon* species arranged according to their geographical distribution, forming three groups of species: Magellanic, Circumantarctic and Insular ones. Probably, because of the particular reproductive biology (embryos develops inside the egg-capsule without plancktonic larvae) the distribution of the species are truly restringed. There is no interchange between Patagonia and Antarctica species. Several species are endemic (*e.g. Trophon macquariensis*, *T. albolabratus* etc). Others have an extensive distribution as *T. geversianus*. However, no Antarctic species occurs in Patagonia and viceversa

Concluding remarks

Kool (1993) demonstrated that *Trophon geversianus* (Pallas, 1774) is closely related to *Nucella lapillus* and *Ocenebra erinacea*. He suggested that this relationship is even closer within this two taxa and the type species of Trophoninae (*T. geversianus*) than with other representatives included in the subfamily. He already suspected that Trophoninae is not a monophyletic group and some of its members may belong to a new group. *Trophon geversianus* (Pallas, 1774) and most of the Patagonian representatives of the subfamily (around 10 species) are closely related among them. Radulae and anatomical features here depicted give support to this statement. Furthermore, living Trophoninae from Patagonia form a compact and probably monophyletic group with a generous amount of fossils representatives.On the other hand, the Antarctic species hitherto included in the subfamily Trophoninae show several features that in the near future could granted a different systematic position.

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Appendix 1. Proposed species names under the genus *Trophon* for South America and Antarctica

1. *acanthodes* Watson, 1882
2. *accuminatus* Strebel, 1904
3. *aculeatus* Watson, 1882
4. *acuminatus* Strebel, 1904
5. *albidus* Philippi, 1846
6. *albolabratus* Smith, 1875
7. *albus* Strebel, 1904
8. *amettei* Carcelles, 1946
9. *antarcticus* Philippi, 1868
10. *brevispira* Martens, 1885
11. *bruceana* Melvill & Standen, 1916
12. *brucei* Strebel, 1904
13. *buccineus* Lamarck, 1816
14. *bulbosa* Perry, 1811
15. *cancellarioides* Reeve, 1847
16. *cancellinus* Philippi, 1845
17. *candidatus* Mabille & Rochebrune, 1889
18. *cinguliferus* Pfeffer, 1887
19. *clenchi* Carcelles, 1953
20. *condensatus* Hedley, 1916
21. *corrugatum* Reeve, 1848
22. *coulmanensis* Smith, 1907
23. *coulmanensis multilamellatus* Numanami, 1996
24. *couthouyi* Strebel, 1904
25. *cribellum* Strebel, 1908
26. *crispus* Gould, 1849
27. *crispus* var. *burwoodianum* Strebel, 1908
28. *cuspidarioides* Powell, 1951
29. *declinans* Watson, 1882
30. *decolor* Philippi, 1845
31. *dispar* Mabille & Rochebrune, 1889
32. *dispectata* Dell, 1990
33. *distantelamellatus* Strebel, 1908
34. *drygalskii* Thiele, 1912
35. *echinolamellatus* Powell, 1951
36. *elegans* Strebel, 1904
37. *elongatus* Strebel, 1904
38. *enderbyensis* Powell, 1957
39. *falklandicus* Strebel, 1908
40. *fasciculatus* Hombron & Jacquinot, 1854
41. *fenestratus* Strebel, 1904
42. *fimbriatum* Martyn, 1784
43. *fimbriatus* Hupé in Gay, 1854
44. *foliaceum* Chemnitz, 1780
45. *foliaceus minor* Chemnitz, 1784
46. *foliatus* Schumacher, 1817
47. *geversianum* Pallas, 1774
48. *geversianus* var. *calva* Kobelt, 1878
49. *gouldi* Cossmann, 1903
50. *gracilis* Perry, 1811
51. *gradata* Ihering, 1897
52. *hoylei* Strebel, 1904
53. *hupeanus* Ihering, 1907
54. *iarae* Houart, 1998
55. *inflatus* Hombron & Jacquinot, 1854
56. *intermedius* Hupé in Gay, 1854
57. *laciniatum* Martyn, 1784
58. *lamellosus* Gmelin, 1791
59. *lebruni* Mabille & Rochebrune, 1889
60. *leptocharteres* Oliver & Picken, 1984
61. *liratus* Couthouy in Gould, 1849
62. *loebbeckei* Kobelt, 1878
63. *longstaffi* Smith, 1907
64. *macquariensis* Powell, 1957
65. *magellanicus* Gmelin, 1791
66. *malvinarum* Strebel, 1908
67. *mawsoni* Powell, 1957
68. *minutus* Melvill & Standen, 1907
69. *mucrone* Houart, 1991
70. *muriciforme* King & Broderip, 1832
71. *necocheanus* Ihering, 1907
72. *nucelliformis* Oliver & Picken, 1984
73. *obesus* Strebel, 1904
74. *ohlini* Strebel, 1904
75. *orbignyi* Carcelles, 1946
76. *ornatus* Strebel, 1904
77. *paessleri* Strebel, 1904
78. *paessleri* var. *turrita* Strebel, 1904
79. *pallidus* Broderip, 1833
80. *patagonicus* d'Orbigny, 1839
81. *pelecetus* Dall, 1902
82. *pelseneeri* Smith, 1915
83. *peruvianus* Lamarck, 1816
84. *philippianus* Dunker in Kobelt, 1878
85. *plicatus* Lightfoot, 1786
86. *plumbeus* Gould, 1852
87. *poirieria* Powell, 1951
88. *priestleyi* Hedley, 1917
89. *pseudoelongatus* Strebel, 1904
90. *ringei* Strebel, 1904
91. *roseus* Hombron & Jacquinot, 1854
92. *scolopax* Watson, 1882
93. *scotianus* Powell, 1951
94. *septus* Watson, 1882
95. *shackletoni* Hedley, 1911
96. *shackletoni paucilamellatus* Powell, 1951
97. *standeni* Strebel, 1904
98. *textiliosus* Hombron & Jacquinot, 1854
99. *unicarinatus* Philippi, 1868
100. *varians* d'Orbigny, 1839
101. *ventricosus* Molina, 1810
102. *veronicae* Pastorino, 1999
103. *verrillii* Bush, 1893
104. *violaceus* Mabille & Rochebrune, 1889

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On the taxonomy and biology of *Chauvetia mamillata* (Risso, 1826) (Gastropoda: Buccinidae) in South East Spain

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KEY WORDS: *Chauvetia mamillata*, Buccinidae, life history, oophagy, *Posidonia oceanica*, *Mesophyllum lichenoides*, "Cabo de Gata-Níjar" Natural Park, Almería, SE Spain.

ABSTRACT The taxonomy, annual cycle, egg capsules, type of development and feeding habits of *Chauvetia mamillata* (Risso, 1826) were studied in three localities of the coasts of Almería (SE Spain), from samples of *Posidonia oceanica* meadows and the coralline alga *Mesophyllum lichenoides*.

Two colour shell phenotypes of this species were observed, one uniformly reddish-brown to dark-brown, and the other pale-brown with darker nodules, but all other studied characters (protoconch, soft parts, radula, egg capsules) showed no significative differences. Furthermore, the life cycles of both phenotypes were coincident. Thus, the two morphs are considered here to be conspecific. We propose the name *Chauvetia mamillata* (Risso, 1826) as the right name for the studied species, being *Chauvetia submamillata* (Bucquoy, Dautzenberg & Dollfus, 1882) a senior synonym. On the other hand, we consider *Chauvetia brunnea* (= *Chauvetia minima*) a different common Atlantic species that reaches the Alborán Sea.

Chauvetia mamillata was present throughout the year in the samples, with a main hatching period in late summer and autumn. The development is intracapsular, without a pelagic larval phase. As a consequence, this species showed a patchy distribution, being abundant in some localities and scarce in others.

Chauvetia mamillata showed nocturnal activity, feeding on egg capsules of other gastropods. It has been observed in aquarium feeding on encapsulated embryos and larvae of *Nassarius incrassatus*, a common species that lives in the same habitats. It is the first known oophagus species of the family Buccinidae.

RIASSUNTO Sono state studiate la tassonomia, il ciclo annuale, le capsule ovigere, il tipo di sviluppo e le abitudini alimentari di *Chauvetia mamillata* (Risso, 1826), in tre località della costa di Almería (SE Spagna), da campioni sulla fanerogama *Posidonia oceanica* e dall'alga corallina *Mesophyllum lichenoides*.

Due fenotipi cromatici sono stati osservati, uno con conchiglia uniformemente da rossiccio-marrone a marrone scuro, l'altro con conchiglia marrone pallido con noduli più scuri. Per tutti gli altri caratteri studiati (protoconca, parti molli, radula, capsule ovigere) non hanno rivelato differenze, e i due morfotipi devono quindi essere considerati conspecifici. Proponiamo il binomio *Chauvetia mamillata* (Risso, 1826) quale corretto nome, con *Chauvetia submamillata* (BDD, 1882) quale sinonimo seniore. D'altro canto, consideriamo *Chauvetia brunnea* (=*Chauvetia minima*) come specie distinta, Atlantica, che raggiunge il mare di Alboran.

Chauvetia mamillata è risultata presente in tutto l'anno nei campioni, con un periodo principale di schiusa nella tarda estate e in autunno. Lo sviluppo è intracapsulare, senza una fase larvale pelagica. Come conseguenza, questa specie mostra una distribuzione a chiazze, essendo abbondante in alcune località e scarsa in altre.

Chauvetia mamillata ha attività notturna, e si nutre di capsule ovigere di altri gasteropodi. Inoltre è stata osservata in acquario, alimentandosi di embrioni incapsulati e larve di *Nassarius incrassatus*, una specie comune che vive negli stessi habitat. Si tratta del primo caso noto di oofagia nei Buccinidae.

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INTRODUCTION

During the years 1986 to 1988, a survey on the molluscan taxocoenosis of the *Posidonia oceanica* (Linné) Delile meadows and concretions of the crustose coralline alga *Mesophyllum lichenoides* (Ellis) Lemoine was made in Los Genoveses, Punta de Loma Pelada and Roquetas de Mar (Almería, SE Spain). All living specimens of molluscs were studied quantitatively (Hergueta, 1996), to analyse these assemblages and the annual cycles of some common species. A small buccinid gastropod of the genus *Chauvetia* was one of the most abundant species in both the *Posidonia* beds and the concretions of *Mesophyllum lichenoides*. Besides, it is one of the most common species on hard substrates and in *Posidonia oceanica* meadows along the southeastern coast of Spain.

Two shell colour phenotypes of this species were present in the samples and are also commonly found in SE Spain, one of them uniform reddish-brown to dark-brown, and the other pale-brown with darker nodules. The first of these phenotypes has been usually named *Chauvetia brunnea* (Donovan, 1804) or *Chauvetia minima* (Montagu, 1803) and the second one has often been named *Chauvetia mamillata* (Risso, 1826) or *Chauvetia submamillata* (Bucquoy, Dautzenberg & Dollfus, 1882).

Despite the reviews of the genus *Chauvetia* in the European coasts done by Nordsieck (1976) and Micali (1999), the taxonomic status of some common littoral species of this genus is still confuse, and some questions remain, like the identity of the

above mentioned four taxa. A thorough revision of the entire genus, taking into account characters such as the colour pattern of the living animal, anatomy, radula, protoconch, egg capsules and development has not yet been attempted, and also a further genetic study would prove very useful.

The objective of this paper is to clarify the taxonomical status of the two common colour phenotypes of *Chauvetia* found in our samples, and to describe the life history and life cycles in the studied localities and habitats.

MATERIAL AND METHODS

The *Posidonia oceanica* meadows of Los Genoveses, Punta de Loma Pelada and Roquetas de Mar and concretions of *Mesophyllum lichenoides* of the last two localities (Fig. 1) were sampled at morning using SCUBA diving, during two or one annual cycles (from march 1986 to march 1988: Tab. 1), depending on the sampling station. The depths at which the samples were taken varied from –0.8 to –3 m in Roquetas de Mar, –3 to –6 m in Los Genoveses, and from –14 to –20 m in Loma Pelada. All Roquetas concretions were attached to *Posidonia* rhizomes, while Loma Pelada concretions were more isolated, and sometimes found on stones near *Posidonia*.

Samples of 30 x 30 cm (900 cm²) with a minimum of 20 cm height of rhizomes were taken in *Posidonia* beds, and concretions of *Mesophyllum* were sampled over 20 x 20 cm (400 cm²). All the samples were first studied "in situ" and after putting them in plastic buckets with seawater. The gastropods were taken out as soon as they ascended to surface due to the anoxic conditions. The remaining sample was fixed in 70% ethanol, and then fragmented concretions, leaves and rhizomes of *Posidonia* were washed over a sieve column of 2, 1, and 0.5 mm; these three fractions were later observed under stereomicroscope to extract the mollusks. Concretions and leaves and rhizomes of *Posidonia* were then separated in order to obtain their respective dry weights (105 ºC during 48 hours).

Table 1. Dates of sampling for each one of the localities. Abbreviations: **CR, PR**= concretions and *Posidonia* from Roquetas de Mar; **CLP, PLP**= concretions and *Posidonia* from Punta de Loma Pelada; **PG**= *Posidonia* from Los Genoveses. Months of June-1987 and February-1988 were not sampled because of bad weather.

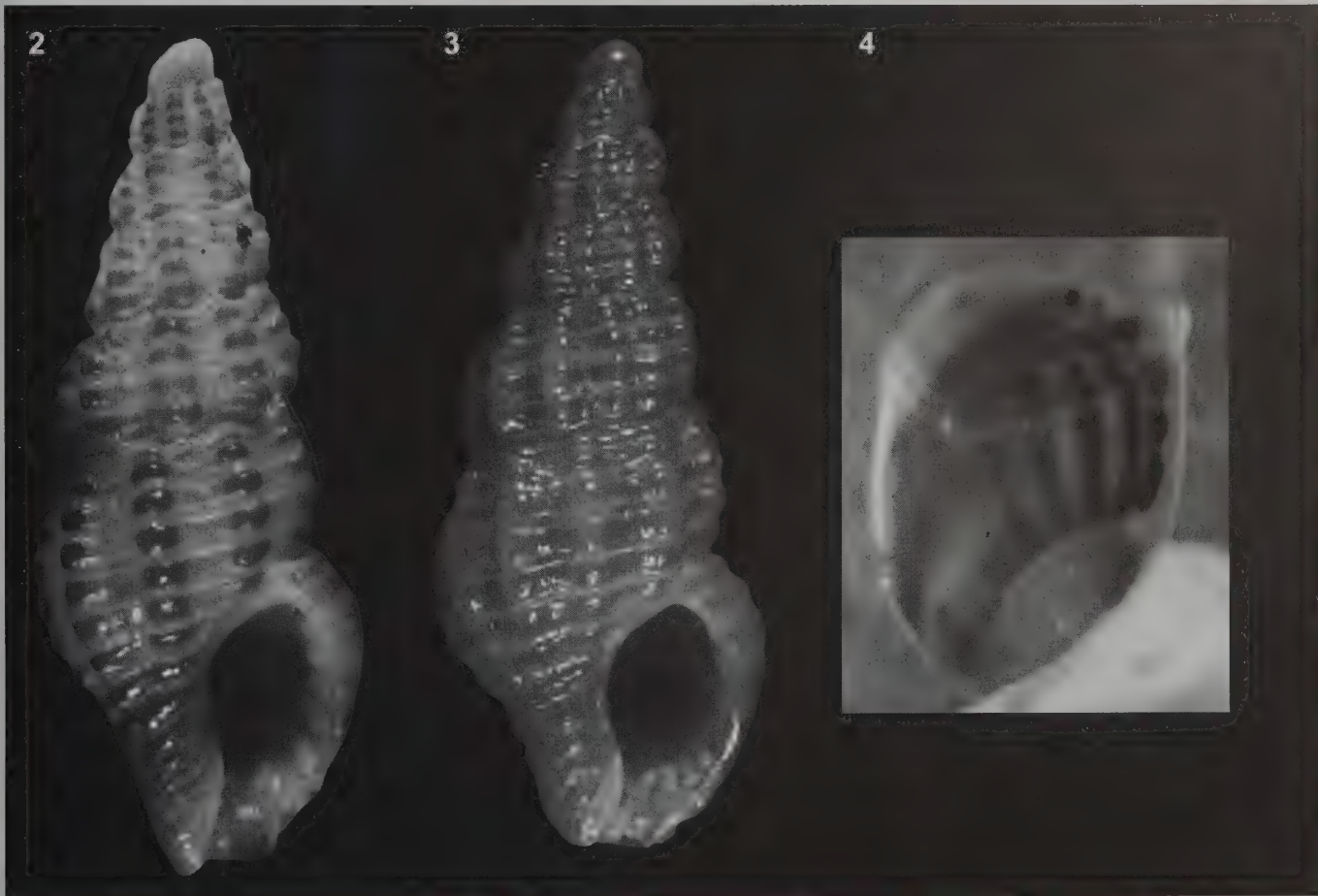
YEAR	MONTH	CR	CLP	PR	PLP	PG
1986	*March*	●				●
	April	●				
	May	●				●
	June	●				
	July	●			●	●
	August	●				
	September	●	●		●	●
	October	●				
	November	●	●			
	December	●				
1987	*January*	●	●		●	●
	February	●	●		●	●
	March	●				
	April	●	●	●	●	●
	May	●		●		
	July	●		●		
	August	●	●	●	●	●
	September	●		●		
	October			●	●	●
	November	●		●		
	December	●	●	●	●	●
1988	*January*	●		●		
	March	●		●		

Figure 1. Map of the southeast coast of Spain, showing the location of the three sampling localities.

Table 2. Number of samples (**N**), total dry weight/habitat (**DW_t**), sample average weight/habitat (**Dw_a**) and minimum and maximum dry weights in grams (**MinDW** and **MaxDW**) for each one of the sampling localities. The values with ± show the average standard error. Rest of abbreviations as in table 1.

	CR	PR	CLP	PLP	PG	Total
N	22	10	7	9	11	59
DW_t	17984.8	7849.2	2567.5	6742.4	12616.5	47760.4
DW_a	817.5±62.5	784.9±53.0	366.8±98.6	749.2±103.6	1147.0±79.2	796.0
MinDW	393.8	464.3	114.5	418.9	753.8	
MaxDW	1333.0	1023.9	625.1	1415.8	1643.6	

Because of the abundances of living specimens in samples may change with different dry weights (furthermore of temporal variations and chance), we use the number of specimens in each sample relating to the average dry weight of all samples (*Posidonia* and concretions), which was 796 g (Tab. 2), getting a new data series calling for us "ponderate abundances". So, each of the ponderate abundance values is a function of the sample dry weight and number of living specimens captured, respect 796 g, and we assume an abundance variation behaviour with a direct proportionality to dry weight, at least within of the range of dry weights registered for our samples. This methodology permitted to compare samples of the same or different habitats, and it has been applied to other groups of invertebrates in similar samples by various authors (García-Raso & Fernández-Muñoz, 1987; Fernández-Muñoz & García-Raso, 1987; García-Raso, 1988; Salas *et al.*, 1988).

Frequency and dominance of *Chauvetia mamillata* were studied using indexes of constancy and dominance like those of previous similar papers (Salas, 1984; Salas & Hergueta, 1986; García-Raso & Fernández-Muñoz, 1987; Martín-Sintes *et al.*, 1987). Constancy index (Ci_x) is defined as $Ci_x = (ni_x/nt_x) \cdot 100$, where Ci_x is the constancy index for the species **i** in the sampled locality **x**, ni_x is the number of samples in the locality **x** in which the species **i** appeared, and nt_x is the number of samples studied in the same locality.

Figures 2-4. *Chauvetia mamillata* (Risso, 1826). **2.** Shell of light phenotype. **3.** Shell of dark phenotype. **4.** Egg-capsule containing a prehatching snail.

Dominance index is defined as $\mathbf{Di_x}$= ($\mathbf{fi_x}$/$\mathbf{ft_x}$)•100, where $\mathbf{Di_x}$ is the dominance index for species **i** in the sampled locality **x**, $\mathbf{fi_x}$ is the number of individuals of species **i** found in locality **x**, and $\mathbf{ft_x}$ is the total number of molluscs found in the station **x**.

Both indexes are equivalent to those used by GLEMAREC (1969) and DAJOZ (1971, *in* MORA, 1980), and they have received different names in literature (e. g. the constancy index as frequency index, and the dominance index as general mean dominance: SOYER, 1970; MARTÍN-SINTES *et al.*, 1987).

According to our own results, the species were classified following the categories established in preceding papers (SALAS & HERGUETA, 1986), and the scale of values used is comparable to those used by other authors (GLEMAREC, 1969; SOYER, 1970; MORA, 1980; TEMPLADO, 1982; GARCÍA-RASO, 1988):

Rare	$Ci_x \leq 15\%$	Rare	$Di_x \leq 0.10\%$
Uncommon	$16\%\ Ci_x \leq 30\%$	Scarce	$0.11\%\ Di_x \leq 0.50\%$
Common	$31\% Ci_x \leq 50\%$	Frequent	$0.51\%\ Di_x \leq 1.00\%$
Very common	$51\% Ci_x \leq 75\%$	Abundant	$1.01\%\ Di_x \leq 2.00\%$
Constant	$Ci_x \leq 76\%$	Dominant	$Di_x = 2.01\%$

Additional material and data of *Chauvetia mamillata*, obtained in *Posidonia oceanica* meadows of Cabo de Palos (Murcia, SE Spain), were also used for comparisons, and to provide information about nycthemeral rhythms. This material comes from the Ph. D. Thesis of the third author (TEMPLADO, 1982).

RESULTS AND DISCUSSION

A total of 1601 specimens of *Chauvetia* were obtained throughout the entire sampling period from the three localities studied in Almería.

Description

Both colour phenotypes have a similar shell form (Figs 2, 3). It is rather small (normally 5-6 mm long in adult specimens, but may reach up to 8 mm), solid, ovoid-fusoid, with a high spire, rather small aperture with a short but distinct siphonal canal. There are approximately 5 slightly tumid whorls with incised sutures. Body whorl about 55% of height. Aperture oval, and nearly 40% of height. Outer lip somewhat thickened, with 5 internal teeth. The sculpture includes 9-12 broad axial ribs, and 4 flattened spiral cords per whorl (8-9 in the body whorl) that are broader than the interspaces.

The shell colour pattern of the light phenotype is pale brown or light-dunnish background colour with reddish-brown spiral cords or granules where they cross the axial ribs. The dark phenotype has an uniform chestnut-brown shell. The dark phenotype seems to be slightly more slender than the light one, but a morphometric study is necessary to confirm this. Some specimens are not easily assigned to one phenotype or another, being useful to observe them immersed in alcohol, because the background colour of the light phenotype is better observed than in dry condition.

The abundance and percentage of each phenotype in the samples from each habitat and locality are shown in Tab. 3. Of the 1601 specimens obtained, 991 (= 61.9%) belonged to the light phenotype and 610 (= 38.1%) to the dark one. This difference in abundances is significant (α= 0.01), and therefore not due to random of the samples.

The protoconch (Figs 5-8) was similar in both phenotypes. It was blunt, paucispiral, with somewhat more than one whorl, 600-700 µm in diameter and 550-680 µm in height. Sculpture consisted of about 22 spiral ridges cross-linked by very fine axial ones, and some broad axial ribs in the final part of the protoconch. A pronounced mark reflecting the hatching event separated protoconch from teleoconch. The lack of differentiation between protoconch I and II would indicate the absence of a planktotrophic larval phase.

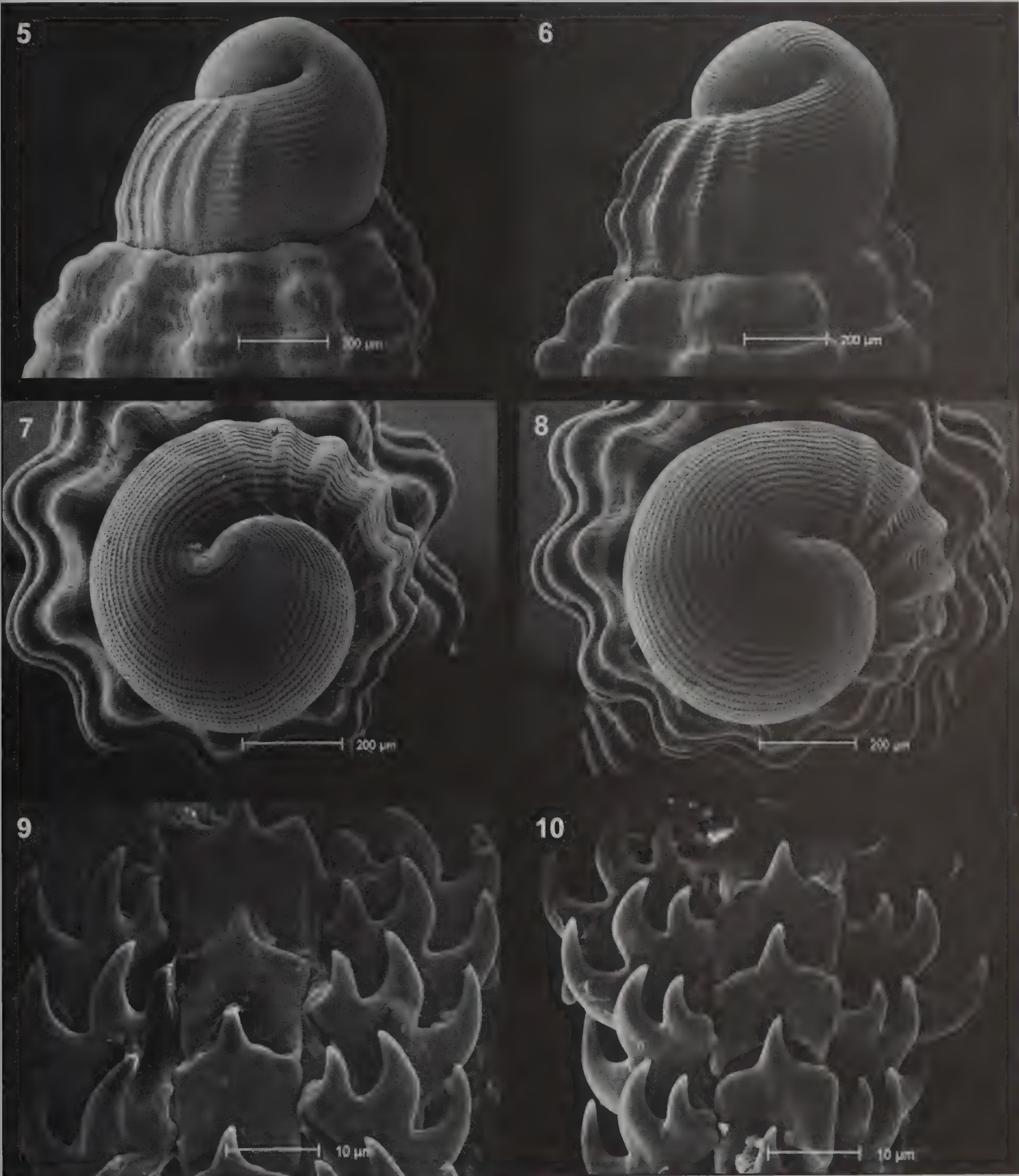
The head-foot in both phenotypes was nearly black, with bluish iridescence, and the sole of the foot was paler. The cephalic tentacles were greyish, with the apical part semitransparent. Sometimes the anterior part of the foot was whitish and some albino specimens were observed, albeit infrequently (11.29% from a sample of N= 62 individuals). Males had a small penis on the right-side of the body. The morphology of the soft parts coincided with that described by FRETTER & GRAHAM (1984) and GRAHAM (1988) for *Chauvetia brunnea* (Donovan, 1804), but the colour pattern differed (GRAHAM, 1971 and FRETTER & GRAHAM, 1986 reported a cream colour with opaque white spots, while GRAHAM, 1988 described a pale brown or tawny pattern with numerous white spots).

The radula was almost identical in both phenotypes (Figs 9-10). The central tooth was nearly quadrangular in outline, with a somewhat concave anterior margin and a central pointed cusp in the posterior one. The lateral tooth had three large and somewhat curved cusps, the outermost being the largest and the innermost the smallest. The radular morphology agreed well with the drawing of the central and lateral teeth of THIELE (1929, fig. 357, as *Chauvetia mamillata*) and the description and SEM micrographs of BANDEL (1977, as *Chauvetia minima*).

Egg-capsules of this species (Fig. 4) were found within concretions of *Mesophyllum lichenoides*. They were cup-shaped (instead of lens-shaped as has been described in *Chauvetia brunnea* by LEBOUR, 1937), with a very short and broad basal stalk. The upper plate constituted the escape aperture, and was sur-

Table 3. Abundance as number of specimens and relative frequency (between parenthesis) of each phenotype of *Chauvetia mamillata* in the habitats and localities sampled . Abbreviations as in table 1.

	CR	PR	CLP	PLP	PLG
Dark phenotype	382 (53.7)	161 (46.9)	2 (2.5)	19 (12.8)	46 (14.5)
Light phenotype	329 (46.3)	182 (53.1)	79 (97.5)	129 (87.2)	272 (85.5)
Total	711	343	81	148	318

rounded by a distinct rounded ridge. The capsules measured nearly 1 mm in height and about 0.9 mm in diameter. Each capsule contained a single embryo that hatched as a crawling juvenile. Thus, as the characters of the protoconch suggest, the larval development is completely intracapsular, without a dispersal larval phase. This kind of development in this species

Figures 5-10. *Chauvetia mamillata* (Risso, 1826). 5, 7. Protoconch of light phenotype. 6, 8. Protoconch of dark phenotype. 9. Radula of light phenotype. 10. Radula of dark phenotype.

was also suggested by TEMPLADO (1982, as *Chauvetia minima*) given the patchy distribution observed in SE Spain, where it was abundant in some localities and scarce in others.

Taxonomy and nomenclature

Despite the differences in the shell colour pattern showed by the two described phenotypes, all other characters studied (protoconch, soft parts, radula, egg capsules) were very similar. Furthermore, both phenotypes live in the same habitats and localities and their life cycles, which were analysed separately, were coincident (HERGUETA, 1996). Thus, the two morphs are considered here to be conspecific. The dark phenotype has been commonly named *Chauvetia brunnea* (Donovan, 1804) or *Chauvetia minima* (Montagu, 1803) in the Mediterranean. The light (or bicolour) phenotype has been often named *Chauvetia mamillata* (Risso, 1826) or *Chauvetia submamillata* (Bucquoy, Dautzenberg & Dollfus, 1882). We propose that the correct name for the studied species is *Chauvetia mamillata* (Risso, 1826), being *Chauvetia submamillata* (Bucquoy, Dautzenberg & Dollfus, 1882) a senior synonym. Due to the different colour of the soft parts, we consider *Chauvetia brunnea* (= *Chauvetia minima*) to be a different common Atlantic species whose range extends beyond the Mediterranean, reaching the Alborán Sea. In contrast, *Chauvetia mamillata* seems to be an exclusively Mediterranean species. The status of the taxon *Chauvetia turritellata* (Deshayes, 1835) could be further clarified in the light of all these characters. According to MICALI (1999), this species differs from *Chauvetia mamillata* by its somewhat smaller protoconch, relatively smaller aperture, greater number of axial ribs and different colour pattern of the soft parts, which have an irregular pattern of dark pigmentation over a white background body colour.

The presence of two clearly different colour phenotypes within the same populations of a single species for the habitats studied here has been also described in *Alvania nestaresi* Oliverio and Amati, 1990 by HERGUETA (1996), and *Bittium reticulatum* (Da Costa, 1778) by MORENO (1998).

Abundance in *Posidonia* meadows and concretions of *Mesophyllum*

The average of the ponderate abundances in each habitats and localities varied between 18.72 and 37.38 individuals (Tab. 4), being Roquetas the locality with the highest population density. Temporal variations and random logically affect the width of the confidence intervals. These results suggest that both the *Posidonia* meadows and the concretions of *Mesophyllum* are very suitable habitats for *Chauvetia mamillata*, and that the similar levels of abundance may reflect similar ecological conditions for both habitats.

Statistical comparison among all the ponderate abundances averages of all localities (Tab. 5) shows that Roquetas is significantly more suitable for *Chauvetia mamillata* than the remaining localities studied (95% of confidence), except for the non significant datum found between *Posidonia* meadows and concretions of Roquetas with concretions from Punta de Loma Pelada: here no differences should be due to random or because the last habitat show small and scarce concretions, with low dry weights (lesser than Roquetas), being therefore a meeting point for molluscs and so the samples reach high ponderate abundances, like both Roquetas formations. Nevertheless, it seems to exist a bathymetrical selection of both habitats by *Chauvetia mamillata*, since this species shows a higher abundance in shallow water samples. This agrees with the higher abundances of this species also found by HERGUETA & SALAS (1987) in similar shallow water concretions of *Mesophyllum* from El Palmer (Almería), established on a *Posidonia* meadow at depths from 2 to 4 m, and where this species became a dominant gastropod.

The similar values of abundance obtained for *Posidonia* meadows of Loma Pelada and Los Genoveses samples, with no significant differences between averages, suggest an almost homogeneous and continuous population. This idea is also supported by the fact that they are neighbouring localities and there are intermediate and probably interconnecting meadows between them.

Chauvetia mamillata does not seem to have specific preferences for *Posidonia* meadows or *Mesophyllum* concretions, since the mean abundances do not significantly differ between the two habitats at the same locality, viz. Roquetas or Punta de Loma Pelada (Tab. 5). In fact, within these localities, it is likely that individuals from both *Posidonia* and *Mesophyllum* belong to the same population, and this will suppose an average of the ponderate abundances of 34.20±6.36 individuals in Roquetas and 19.97±9.47 in Punta de Loma Pelada (in both cases, values are given for a 95% confidence interval).

Temporal variations of abundance

The two-year study of the concretions of Roquetas shows the variation of abundance through time. In this habitat, the population maintained constantly high values of abundance through the two annual cycles, since we did not found significant differences among the mean values of the two series of ponderate abundances, which were 33.88±11.05 and 31.41±14.58 individuals (α= 0.05), respectively, for the first and the second years. Thus, we assume a stable population with little intra and interannual variations during the period studied.

The monthly variations of ponderate abundance series (considering both phenotypes together) in Roquetas (Fig. 11) show temporal fluctuations, which do not allow us to establish a clear trend. *Chauvetia mamillata* seems to increase irregularly its population in different times of the year, in a non repetitive way along the two cycles and apparently at random. Notwithstanding, there is certain parallelism between populations from concretions in the second year and *Posidonia* bed samples from Roquetas, which may reflect a similar evolution of the species in both habitats. HERGUETA (1996) reported abundance cycles with a similar behaviour for each phenotype.

The possible periodic fluctuations of ponderate abundance series of whole specimens (including both phenotypes) were analyzed in concretions from Roquetas, by mean of a correlation analysis based on Pearson's coefficient r product-moment (according to SOKAL & ROHLF, 1986). Only those correlations with r higher than the critical value has been considered, and are represented in Fig. 12 for confidence limits of 95 and 99%, since these significant points reflect a regularity non due to ran-

dom. A significant periodicity has been observed corresponding to 12 diphases, and it points out to an almost annual cyclic rhythm of abundance, like those of several other species of small gastropods from these habitats studied by HERGUETA (1996).

Chauvetia mamillata is also present throughout the year in the concretions of *Mesophyllum* associated with *Posidonia* studied by HERGUETA (1985) in Nerja (Málaga, S. Spain), but in this locality a trend to higher abundances in winter and an annual maximum in spring were observed. In contrast, TEMPLADO (1982) recorded low abundances in spring and highest abundances in August-September in *Posidonia* meadows from Cabo de Palos (Murcia).

In the remaining habitats and localities that comprise this survey, *Chauvetia mamillata* seems to have the highest abundance in summer (concretions of Punta de Loma Pelada and Los Genoveses) and autumn (*Posidonia* of the same localities, see Fig. 13). This is probably related to maximum recruitment events (see below).

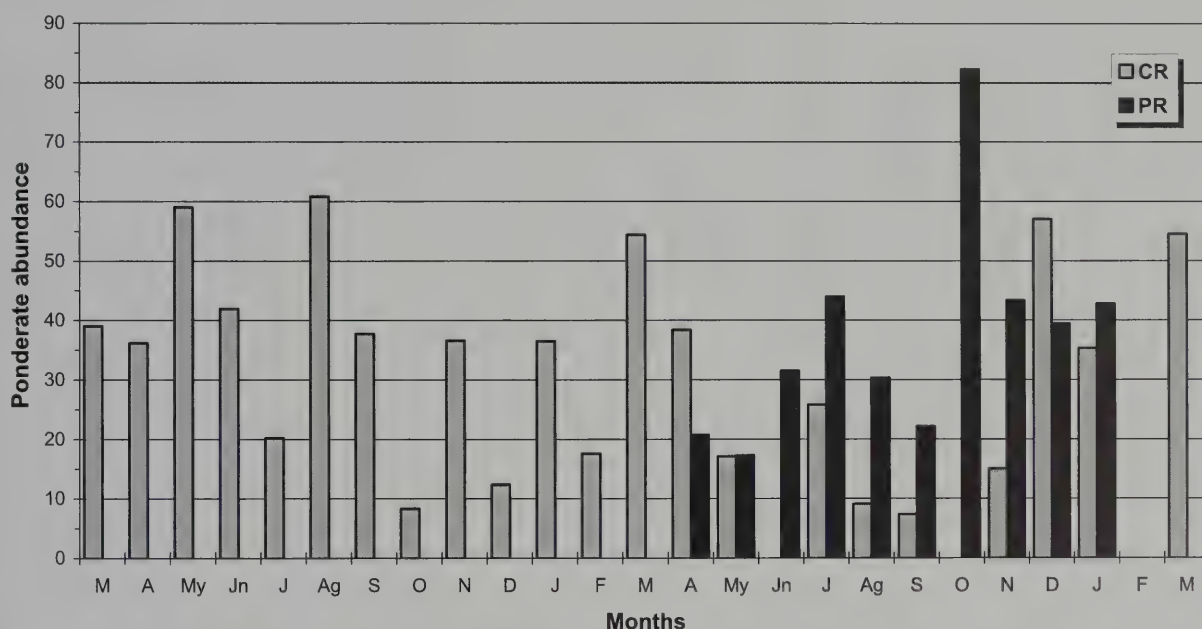



Figure 11. Ponderate abundance cycle of *Chauvetia mamillata* in concretions of *Mesophyllum lichenoides* and *Posidonia oceanica* meadows of Roquetas, through the two years survey. Abbreviations, as in table 1.

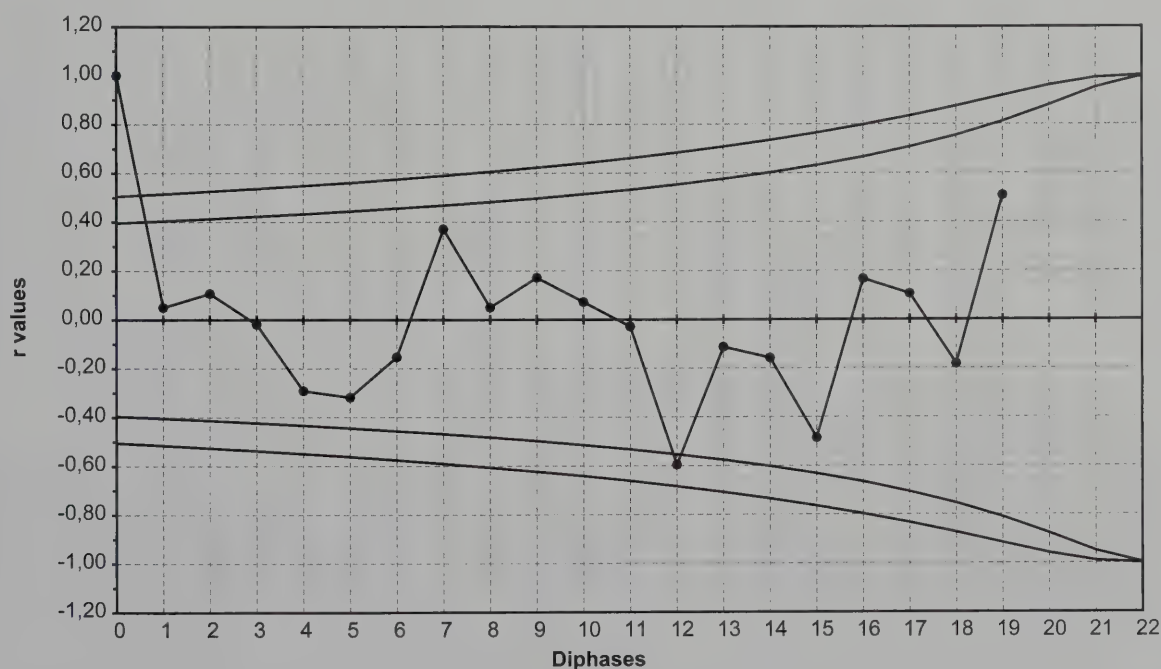



Figure 12. Autocorrelogram of ponderate abundances series of *Chauvetia mamillata* from concretions of *Mesophyllum lichenoides*, Roquetas de Mar. The curved lines near to X-axis represent the 95% limit of significance, and those more far the 99% limit.

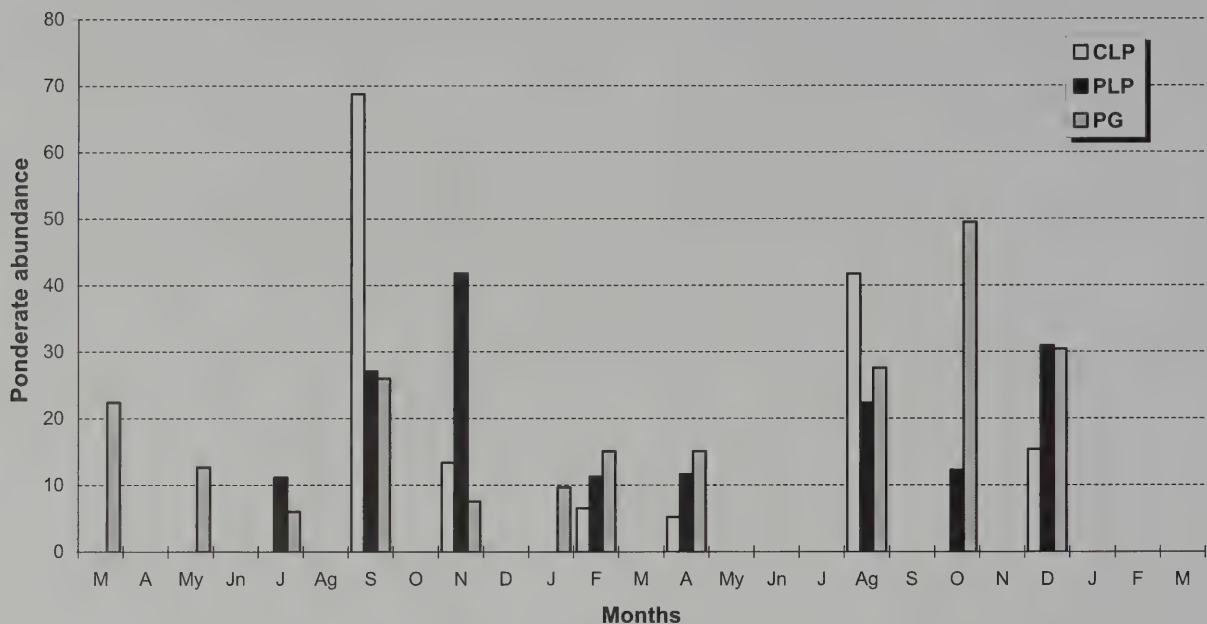



Figure 13. Ponderate abundance cycle of *Chauvetia mamillata* in concretions of *Mesophyllum lichenoides* and *Posidonia oceanica* meadows of Punta de Loma Pelada and Los Genoveses.

Frequency and dominance

Chauvetia minima is a main component of all the studied taxocoenosis, with higher or even maximum indexes of frequency, and higher dominance indexes (Tab. 6).

According to these results and the classificatory criteria described under Material and Methods, *Chauvetia mamillata* should be considered a constant and dominant species for all the localities and habitats studied. Though this species also appeared in other benthic communities in these localities, it was more abundant (preferential) in concretions and *Posidonia* meadows. Thus, this species is one of the dominant species in these habitats, together with *Alvania nestaresi* Oliverio and Amati, 1990, *Alvania tenera* (Philippi, 1844), *Sinezona cingulata* (O. G. Costa, 1861), *Columbella rustica* (Linné, 1758), and *Bittium latreillei* (Payraudeau, 1826) (HERGUETA *et al.*, 1992; HERGUETA, 1996). In Cabo de Palos, TEMPLADO (1982) separately sampled leaves and rhizomes in *Posidonia* meadows, and observed that *Chauvetia mamillata* was also one of the dominant species in both strata, together with *Jujubinus exasperatus* (Pennant, 1777), *Rissoa auriscalpium* (Linné, 1758), *Rissoa ventricosa* Desmarest, 1814, and *Rissoa variabilis* (Von Mühlfeldt, 1824) on the leaves, and with *Alvania cimex* (Linné, 1758), *Columbella rustica* (Linné, 1758), *Clanculus cruciatus* (Linné, 1758) and the bivalves *Striarca lactea* (Linné, 1758) and *Cardita calyculata* (Linné, 1758) on the rhizomes.

Table 4. Average of the ponderate abundances/sample (**Paa**) of *Chauvetia mamillata* (both phenotypes together) in each of the prospected localities. Right values of ± symbol show the 95% confidence interval, with the opportune corrections for N<30. Abbreviations, as in table 1.

	CR	PR	CLP	PLP	PG
Paa	32.76±7.92	37.38±14.04	21.57±24.82	18.72±10.49	20.18±9.01

Table 5. Significance of means of table 4. NS= no significant difference; S= significant difference with a 95% confidence. Abbreviations as in table 1.

	CR	PR	CLP	PLP	PG
CR	-	NS	NS	S	S
PR		-	NS	S	S
CLP			-	NS	NS
PLP				-	NS
PG					-

Table 6. Constancy (Ci_x) and dominance (Di_x) of *Chauvetia mamillata* in the localities and habitats studied. Abbreviations as in Table 1.

Locality	Ci_x (%)	Di_x (%)
CR	100.00	4.76
PR	100.00	5.95
CLP	85.71	4.17
PLP	88.89	4.75
PLG	100.00	6.07

Appearance of egg-capsules and newly hatched young snails

A total of 33 non-hatched egg-capsules were found attached to concretions in Roquetas. No egg-capsules were found in concretions of Punta de Loma Pelada, probably due both to the low number of samples, as well as to its different spatial configuration with respect to Roquetas concretions (smaller size, few cavities, scarce relationship with rhizomes, etc.). No egg-capsules were found in *Posidonia* from the three localities. This may have been due largely to the way in which the leaves and rhizomes were processed in the laboratory, where they were only washed and sieved, and were not directly observed under a stereomicroscope. Thus, *Chauvetia mamillata* may use rhizomes of *Posidonia* for reproduction as much as concretions.

Egg-capsules appeared in Roquetas from April to August 1986, and from March 1987 to January 1988, except in September 1987, when no capsules were found. This suggests a wide reproductive period throughout almost the entire year, with a maximum of 57.1% of total egg-capsules in July of the first year, and 50% in October and November of 1987.

The newly hatched young snails were also present in concretions from Roquetas in almost all the months (Fig. 14), but higher percentages were found from July to September in the first year (56.5% of total), clearly related to the highest abundance of egg-capsules in July, and in October and December of 1987 (64.3% of total), related to the maximum of egg-capsules of October and November. It seems that suitable reproductive periods depend on the years considered, and may be spring-summer or autumn; nevertheless it should not be discarded a continuous reproduction at least along the period from spring to autumn.

The evolution of percentages of newly hatched young snails from *Posidonia* meadows of Los Genoveses shows an almost identical trend to that of concretions from Roquetas (Fig. 15), with presence of postlarvae throughout almost all the period sampled, and highest percentages in summer (first year) and autumn (second year).

These results agree in part with those of TEMPLADO (1982), who found young snails in summer and autumn in *Posidonia* meadows from Cabo de Palos, and HERGUETA (1985), who recorded an autumnal recruitment in concretions of *Mesophyllum lichenoides* from Nerja (Málaga).

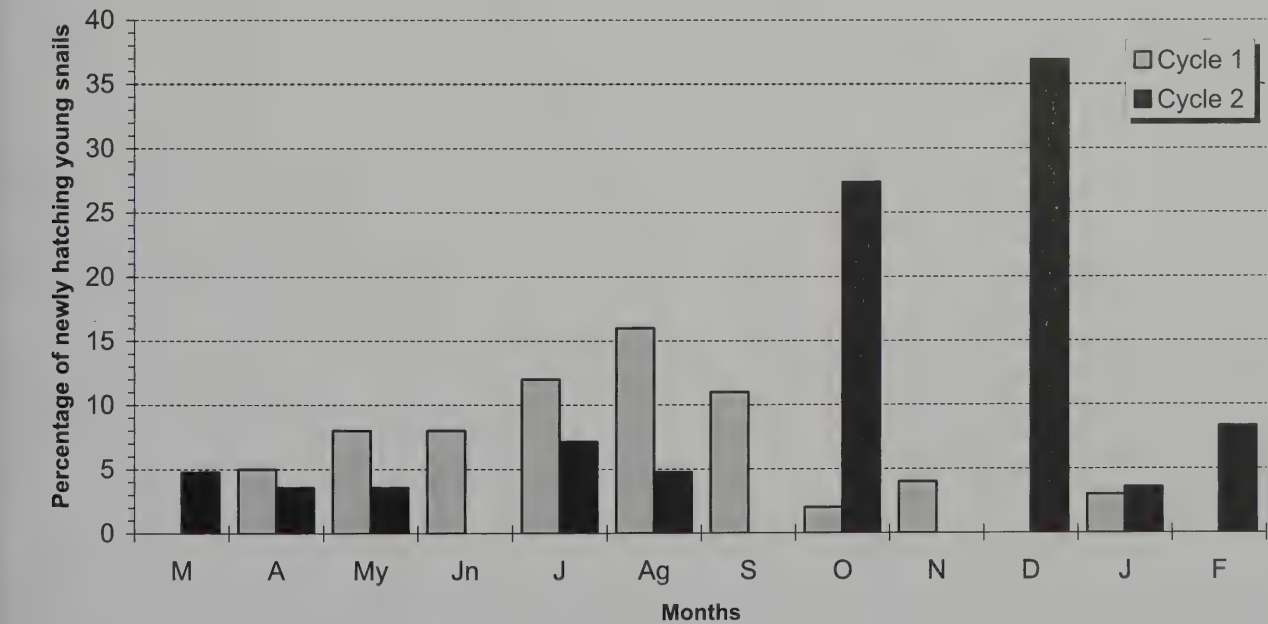



Figure 14. Percentage of newly hatching youngs of *Chauvetia mamillata* in concretions from Roquetas, through the two sampled cycles.

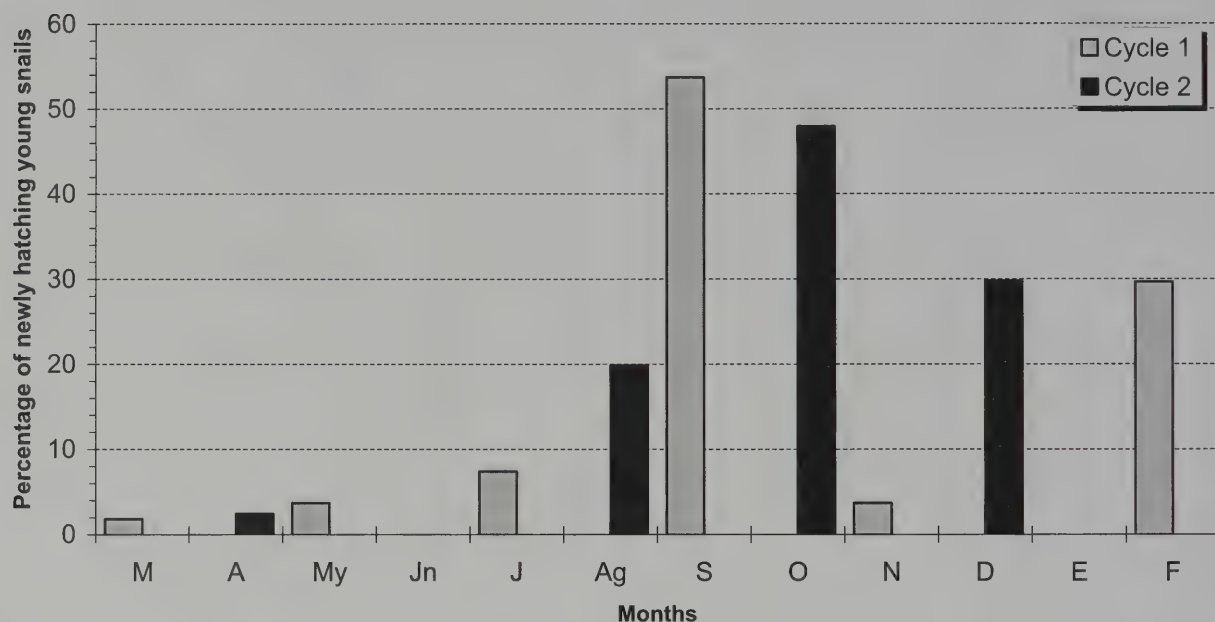



Figure 15. Percentages of newly hatching youngs of *Chauvetia mamillata* in *Posidonia oceanica* meadows from Los Genoveses, throughout the two sampled periods.

Feeding

Chauvetia mamillata shows nocturnal activity, as also pointed out Russo *et al.* (1984). It feeds on egg-capsules of other gastropods, and has been observed in aquaria feeding on encapsulated embryos and larvae of *Nassarius incrassatus*, another very common species that lives in the same habitats. The animal introduces the proboscis into the egg-capsule through the apical escape aperture, and using the radula, extracts the embryos or the soft part of the pre-hatching veligers, leaving the larval shell empty.

Egg masses of many other gastropods are common on the rhizomes and leaves of *Posidonia oceanica* and on concretions of *Mesophyllum lichenoides*. In the samples studied, egg masses of *Pollia dorbignyi* (Payraudeau, 1826) and *Nassarius incrassatus* (Ström, 1768) predominated. The greatest numbers of egg-capsules of the latter species were found in Roquetas in May of both annuals cycles (Hergueta, 1996), starting at February, and no egg-capsules were observed the remaining months. Egg-capsules of *Pollia dorbignyi* seldom appear on thala of *Mesophyllum lichenoides* in March and May, with a maximum in July (Hergueta, 1996). In both species, highest abundances of egg-capsules coincide with highest abundances of adults of *Chauvetia mamillata* (May-June).

Among prosobranch gastropods, up to date oophagy is only known in some species of Columbellidae (Taylor, 1987; Harasewych, 1990; De Maintenon, 1999). Within opisthobranchs, oophagy is known in the aeolid *Calma glaucoides* (Alder & Hancock, 1854), that feeds on fish eggs (Schmekel & Portmann, 1982), and in the sacoglossans *Calliopaea bellula* d'Orbigny, 1837 (= *C. oophaga* Lemche, 1974) and *Olea hansineensis* Agersborg, 1923, both feeding on eggs of other opisthobranchs (Jensen, 1986, 1997). Therefore, our data provide evidence that *Chauvetia mamillata* is the first known oophagous species of the family Buccinidae. Michel & Poulicek (1987) noted that the species *Mitrella scripta* (Linné, 1758) and *Chauvetia affinis* (Monterosato, 1889) (synonym of *Chauvetia turritellata* Deshayes, 1835, according to Micali, 1999) were the most common neogastropods in nests with eggs of the labrid *Symphodus ocellatus* (specially at night), and suggested that fish oophagy seems to be feasible in both species.

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Venomous Gastropods: *Conus*, conoideans and other neogastropod families

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ABSTRACT: A review of the present undestanding of the mechanism of envenomation by cones is presented. The expanding applications of cone snail venom components in biomedical science are the degree to wich the envenomation strategy may be shared by other venomous gastropod groups is explored based on a preliminary molecular phylogenetic analasys. Finally, some perspectives for the future are discussed.

RIASSUNTO: In questo lavoro viene inizialmente presentata una revisione delle conoscenze attualmente disponibili sul meccanismo di uso dell'apparato velenifero da parte delle specie di *Conus*. Quindi viene introdotto il campo in crescente espansione dell'applicazione in medicina dei componenti del cocktail tossinico utilizzato dai coni. Si esplora inoltre, sulla base di un'analisi filogenetica indipendente su base molecolare, la questione del possibile livello di condivisione dei medesimi meccanismi veleniferi in altri gruppi di neogasteropodi. Infine vengono discusse alcune prospettive di studio per il futuro.

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INTRODUCTION

The remarkable biology of cone snails (*Conus*) can be encapsulated by two well-established, if improbable observations. Cone snails are the only known gastropods capable of killing humans, first documented by Rumphius nearly three centuries ago (Rumphius, 1705). In addition, some *Conus* are the only gastropods known to capture fish as major prey, a phenomenon first established by Alan Kohn (Kohn, 1956). Although most cone snail species are not capable of killing humans and do not hunt fish, the fact that some species have evolved these seemingly impossible capabilities highlight the unusual evolutionary directions of the genus.

One evolutionary breakthrough that makes such unusual biology possible is the presence of a complex venom with corresponding anatomical adaptations for venom delivery, including harpoon-like teeth which also serve as hypodermic needles. Recently, cone snail venoms have been the focus of considerable biochemical and physiological investigation. These studies have revealed that this large successful genus (500 species) has evolved a highly sophisticated neuropharmacology.

This paper comprises three sections: first is a review of our present understanding of the mechanism of envenomation by predatory cone snails. The second section introduces an emerging field, the expanding applications of cone snail venom components in medicine. A third section addresses a scientific question not yet incisively addressed: the degree to which the cone snail envenomation strategy may be shared by other venomous gastropod groups. Specifically, how much overlap in mechanism to *Conus* will be found in other toxoglossate gastropods such as the Terebridae and the Turridae? These are the most obvious groups that may have mechanisms similar to the cone snails (family Conidae) since they are conventionally placed either in the same superfamily (Conoidea) or suborder (Toxoglossa) by most taxonomists. As mechanisms underlying cone snail envenomation become increasingly well elucidated at a genetic and molecular level, a comparison between the three major groups of neogastropods that envenomate prey becomes more feasible. In this paper, we discuss the likelihood of overlap between *Conus* and other toxoglossate molluscs, an evaluation based not on a direct characterization of the venoms of the other gastropod groups, but rather on an assessment of relationships between various families within the Neogastropoda.

After the review of *Conus* envenomation, the overview of potential medical applications of *Conus* venom components and the assessment of relationships between the Conoideans and other family groups within the neogastropods, the brief Discussion section includes some perspectives for the future.

I. Overview of *Conus* Envenomation

Conotoxins. The initial biochemical characterization of venoms from several *Conus* species firmly established that the biologically-active principles are small, highly structured polypeptides called conotoxins (alternatively, *Conus* peptides or conopeptides), which potently affect nervous system function by binding to specific molecular targets, primarily ion channels or receptors on the surface of neurons (Olivera et al., 1985a). The majority of conotoxins are neurotoxins between 8-45 amino acids in length. Despite their small size, conotoxins are confor-

mationally relatively rigid - in most cases, the three-dimensional structure is stabilized by multiple intramolecular disulfide crosslinks within the polypeptide (for overviews, see (Olivera et al., 1990; Olivera, 1997)). As a class, conotoxins are the smallest neurotoxins from animal venoms directly encoded by genes.

Since the discovery and characterization of the first conotoxins, an intriguing juxtaposition has emerged. On the one hand, *Conus* venoms have proven to be exceedingly complex. On the average, every cone snail has a venom repertoire of over 100 diverse conopeptides, each encoded by a different gene. On the other hand, there is an underlying simplicity: the great majority of conotoxins found in the ca. 500 different species of cone snails belong to only a few gene superfamilies (Olivera et al., 1999). All conotoxins of a superfamily share conserved sequence features. Thus, although a *Conus* venom is a complex biochemical mixture, several generalizations apply.

The genes encoding conotoxins are expressed in the cells lining the lumen of venom ducts of cone snails. The initial translation products are polypeptide precursors between 80-120 amino acids in length (Woodward et al., 1990). For most conotoxins, multiple post-translational modifications occur, including covalent modification of some amino acids (Craig et al., 1999a) and trimming of the precursor into the mature, biologically-active *Conus* peptide (the majority of which are 12-30 amino acids). Thus, most of the polypeptide precursor is trimmed off as maturation of the biologically-active conotoxin occurs. Conotoxin precursors (with some post-translational modifications) are stored in the venom duct as granules. As the venom transits from the duct through the proboscis to the hollow, harpoon-like radular tooth, a processing cascade to mature conotoxins occurs, very probably involving proteolytic secretions from the proboscis (Olivera et al., 1985b).

After venom is injected by a cone snail, each individual conotoxin probably targets a single molecular component in the nervous system of the injected animal. However, groups of different conotoxins in the same venom may act together towards a common physiological end. Such a synergistic group of venom peptides is called a conotoxin "cabal" (Olivera and Cruz, 2001). One example is the "motor cabal," a group of toxins that inhibits neuromuscular transmission in prey animals. One component of the motor cabal might inhibit release of neurotransmitter, another blocks the neurotransmitter receptor on the muscle, and a third component might inhibit electrical signaling on the muscle membrane. Together, such a group of toxins would efficiently suppress locomotion of the prey. Most cone snails have a motor cabal of conotoxins that rapidly and efficiently cause paralysis in prey. However, there are other functional toxin cabals with different physiological endpoints. For example, a "lightning strike cabal" has been identified in certain fish-hunting cone snail venoms; these elicit a rapid, potent electrical shock-like syndrome from the site of injection, stunning the prey and causing immediate immobilization. Some species (such as the Panamic fish-hunting species, *Conus purpurascens*) have both a "lightning-strike" and a "motor" cabal of toxins (Terlau et al., 1996). It has been suggested that *Conus* species that capture schools of fish using a net strategy have a cabal of peptides in their venom that deadens sensory circuitry, so that the engulfed fish seem sedated; this has been termed the "nirvana cabal" (Olivera and Cruz, 2001).

Divergence of venoms between *Conus* species. A surprising insight arising from the characterization of different *Conus* venoms is the remarkable divergence of conotoxins between cone snail species. Since any cone snail venom can have >100 different components, it might have been expected that a significant fraction would be conserved across all *Conus* species. Instead, it appears that every *Conus* has its own distinct complement of peptides.

This has been established using both biochemical and molecular genetic methods. What has emerged from these studies is that the mature toxin region of conotoxin genes hypermutate rapidly as speciation occurs. In contrast, other sequence elements of conotoxin genes, in particular the exons encoding the signal sequences at the N-terminal end of every conotoxin precursor, are unusually conserved. Thus, at the genetic level there is a striking contrast: one part of a conotoxin gene, the mature toxin region (which is always at the C-terminal end) undergoes hypermutation, while the other end of the translation product (the N-terminal signal sequence) shows an unprecedented sequence conservation (Woodward et al., 1990; Olivera, 1997).

Over the time period relevant for evolution of new *Conus* species, hypermutation at the C-terminal, mature-toxin region provides a mechanism for cone snails to explore many peptide sequences. In essence, the snails have used what is now a state-of-the-art technology for drug development in pharmaceutical companies, the "combinatorial library strategy" for drug development (except that cone snails antedated the large pharmaceutical firms by over 50 million years!).

The highly conserved signal sequences within individual conotoxin gene superfamilies imply a correspondingly conserved cellular secretion and maturation pathway. We postulate that the signal sequences of conotoxin precursors may direct these to particular intracellular membrane loci associated with secretory pathways with appropriate accompanying accessory factors such as post-translational modification enzymes, and possibly, specific chaperone-type proteins for facilitating folding and disulfide bond formation of specific peptide superfamilies.

In essence, the N-terminal regions of conotoxin genes are conserved when two homologous sequences are compared from different species, but focal hypermutation results in a very rapid sequence divergence in the C-terminal mature toxin regions. It was postulated that the large introns characteristic of conotoxin genes may play a role in the differential rates of mutation observed (Olivera et al., 1999); recently, a specific mechanism for hypermutation has been proposed (Conticello et al., 2001).

Thus, the biologically active, mature venom components show an amazing sequence diversity from one species to the next. This appears to be one basis for the evolutionary success of this large group of marine neogastropods, arguably the largest living genus of marine animals (Röckel et al., 1995).

II. Medical and basic neurobiological research applications of *Conus* venom peptides

Although one impetus for investigating conotoxins was the mortality and morbidity caused by cone snail envenomation, an accelerating interest in these peptides stems from what seems to be another improbable juxtaposition: the potential of *Conus* venom components to serve as therapeutic agents. As we discuss below, conotoxins are already being used as diagnostic tools, and for basic biomedical investigations in understanding nervous systems. However, recent research on conotoxins has demonstrated some exciting therapeutic possibilities.

One conotoxin, ω-conotoxin MVIIA, was initially purified and characterized from the venom of the fish-hunting *Conus* species, *Conus* magus, approximately twenty years ago by Michael McIntosh, then an undergraduate at the University of Utah. This compound is now sufficiently far along in terms of drug development that it may be approved this year in the US as a commercial drug under the generic name "ziconotide" (Elan Pharmaceuticals, which will market the drug, has received an "approvable" letter from the U.S. Federal Drug Administration) (McIntosh et al., 1982; Olivera, 2000). The structure of this peptide, originally derived from the venom of *Conus magus* is shown in Table I. The commercial drug, which is being synthesized chemically, is identical in every respect to the natural product.

The proposed therapeutic application of Ziconotide is to alleviate intractable pain syndromes, in particular the malignant pain of cancer patients. The present therapy for intense pain involves opiate drugs such as morphine. Given this, why was it feasible to develop a more complex compound? Ziconotide has a number of disadvantages compared to morphine, most notably in terms of the requirements for drug delivery - Ziconotide cannot be taken orally, and even worse, has to be injected directly into the spinal cord.

Ziconotide targets a particular molecular form of voltage-gated calcium channel, found in all vertebrate nervous systems. In the human spinal cord, this calcium channel isoform is very restricted in its distribution: it is found in synapses between input pain fibers and spinal cord nerve cells which transmit pain signals to the brain. Blocking this synapse blocks transmission of a pain signal to the higher CNS centers (Olivera, 2000); the result is that the patient does not perceive the intense pain that would otherwise manifest itself.

Morphine also helps to block transmission of this signal; however, a major problem with morphine is that if it has to be used repeatedly, patients develop tolerance. This is because

Table I. Example of paralytic conotoxins

I.		
α-conotoxin GI (*Conus geographus*)	E**CC**NPA**C**GRHYS**C**#	Physiological effect: cause paralysis of the fish prey of these piscivorous species by blocking synaptic transmission. Physiological mechanism similar to major neurotoxins found in Cobra and related snake venom
α-conotoxin EI (*Conus ermineus*)	RDO**CC**YHPT**C**NMSNPQI**C**#	Molecular mechanism: antagonists of nicotinic acetylcholine receptor, skeletal muscle subtype
II.		
ω-conotoxin GVIA (*Conus geographus*)	**C**KSOGSS**C**SOTSYN**CC**RS**C**NOYTKR**C**Y#	Physiological effect: blocks neurotransmitter release which results in paralysis of the fish prey of these piscivorous species
ω-conotoxin MVIIA (*Conus magus*)	**C**KGKGAK**C**SRLMYD**CC**TGS**C**RSGK**C**#	Molecular mechanism: antagonists of calcium channels
III.		
δ-conotoxin PVIA (*Conus purpurascens*)	EA**C**YAOGTF**C**GIKOGL**CC**SEF**C**LPGV**C**FG#	Physiological effect: cause paralysis of fish (*C. purpurascens*) and mollusc (*C. textile*) by blocking synaptic transmission
δ-conotoxin TxVIA (*Conus textile*)	W**C**KQSGEM**C**NLLDQN**CC**DGY**C**IVLV**C**T^	Molecular mechanism: delays inactivation of sodium channels

morphine activates a receptor in the spinal cord (the opioid receptor) which intrinsically becomes less sensitive as it is turned on (in pharmacological parlance, it is "down-regulated"). Thus, after repeated use, patients become tolerant to morphine and it becomes increasingly difficult to alleviate their pain. However, for Ziconotide the continual use of the conotoxin does not result in down-regulation of its targeted voltage-gated calcium channel, and patients do not become tolerant to the drug. Thus, cancer patients who have become tolerant to morphine are candidates for Ziconotide therapy. This conotoxin drug has already been through extensive clinical trials in human patients and final approval to market Ziconotide is anticipated in the year 2001.

In addition to Ziconotide, several other *Conus* peptides are being explored for their therapeutic potential. One that has entered clinical trials is a 17-amino acid peptide discovered by Craig Clark, another undergraduate at the University of Utah, which is now called conantokin-G (Olivera et al., 1985b). This peptide is being developed as a drug for cases of intractable epilepsy. The peptide acts as a specific inhibitor of an important central nervous system component known as the NMDA receptor; conantokin-G quiets down overactive neuronal circuitry by inhibiting NMDA receptors. In animal models, the efficacy of the drug compared to its behavioral toxicity seems much better than are existing therapies for epilepsy (White et al., 2000). This compound is being developed by a small biotech company, Cognetix Inc. of Salt Lake City, Utah, in collaboration with a drug-delivery company, Medtronic, Inc. of Minneapolis, Minn.

The two examples above are furthest along in terms of clinical development for therapeutic use. A number of other conotoxins have been tested in animal models and shown to have promise as therapeutic agents. One of the most novel of these peptides is contulakin-G, an O-glycosylated, 17-amino acid peptide from *Conus geographus* which is believed to be a possible agonist of a specific neurotensin receptor subtype in the central nervous system; this has shown promising analgesic properties (Craig et al., 1999b; Wagstaff et al., 2000). Other *Conus* peptides being explored by Cognetix have potential application as local anesthetics, muscle relaxants, and in demyelinating diseases such as multiple sclerosis. Among the other *Conus* peptides being developed is ω–conotoxin CVID as an analgesic (by Xenome, Inc. of Brisbane, Australia) (Lewis et al., 2000; Nielsen et al., 2000; Wright et al., 2000). So far, only a miniscule fraction of the total number of conotoxins have been explored for therapeutic applications; the activity in this area is clearly increasing exponentially as monitored by publications in pharmacological journals, number of patent applications being filed and patents which have issued in the last few years (Jones and Bulaj, 2000; Jones et al., 2001).

Conotoxins also have clear uses in diagnostic medicine; one of the applications which is already well established is the use of radiolabeled ω–conotoxins for evaluating potential patients with the Lambert-Eaton myasthenic syndrome, an autoimmune neurological disorder associated with small cell lung carcinomas (Lennon, 1996; Lang et al., 1998). The radiolabeled peptide is used to determine whether the patient has elevated levels of autoantibodies that may interfere with the proper functioning of voltage-gated calcium channels at the junction between motor nerves and muscle.

Finally, the application of individual conotoxins as basic research tools in neuroscience is now very well established. Many *Conus* peptides have proven to be useful in identifying molecular components in various functional circuits, and indeed in certain cases these peptides are the only agents available for assaying involvement of certain molecular targets. Particularly notable are the use of ω–conotoxins for inhibiting neurotransmitter release (Olivera et al., 1994) and the use of α–conotoxins for identifying nicotinic acetylcholine receptor subtypes (McIntosh et al., 1999). There are now over 2,200 publications in the primary research literature that describe experiments where conotoxins have been employed as basic research tools, In effect, cone snail venom components are being widely used by neuroscientists to understand our own brains.

III. Neogastropod families and the superfamily Conoidea

Background. Recently our laboratories carried out a phylogenetic reconstruction of a large group of *Conus* species (>70) (Espiritu et al., 2001) using mitochondrial 16S RNA sequences. A number of other gastropods were included in this analysis - the original intent was to have these serve as the outgroups for identifying clades of species in the genus *Conus*. As a consequence, sequence data from several different neogastropod families became available. We present the data and the analysis of the mitochondrial 16S ribosomal RNA from six neogastropod families: Conidae, Turridae, Terebridae, Costellaridae, Mitridae, and Olividae (see Fig. 1 for the species analyzed). One mesogastropod from the family Cerithidae, *Rhinoclavis aspera*, is also included here to serve as the outgroup for rooting phylogenetic trees.

The phylogeny of the neogastropods is in flux (for reviews, see (Ponder, 1973; Taylor and Morris, 1988; Kantor, 1996)), and therefore any new molecular data should contribute to the evaluation of the many alternative proposals regarding their phylogeny. Although a general revision of neogastropod phylogeny was not our goal, the preliminary analysis we carried out supports a surprising and unexpected phylogenetic hypothesis that should be examined further by a more comprehensive study.

It has been the general practice to organize families of Neogastropoda into superfamilies (or suborders), implying that the families within a particular superfamily are more closely related to each other than to other neogastropod groups. The neogastropod group of most direct concern to the authors is the venomous superfamily Conoidea (Conacea, or suborder Toxoglossa). Traditionally, three large recent neogastropod families - Conidae, Turridae and Terebridae - are included in the Conoidea. This is one grouping which remains a relatively constant feature of most taxonomic proposals made for the Neogastropoda.

In addition to the Conoidean species, our analysis included species in the families Costellaridae, Mitridae and Olividae (see Fig. 1D). In one of the more recent conventional phylogenies, these are grouped together in the superfamily Muricoidea with many other neogastropod families. In some other taxonomic schemes, these families are assigned to a smaller superfamily, Volutacea. One standard widely used taxonomy for the species analyzed here is shown in Table II.

All conventional phylogenies predict that all neogastropod groups would be more divergent from *Rhinoclavis aspera* than they would be from each other (since mesogastropod and neogastropod families are usually assigned to separate orders of the class Gastropoda). An additional prediction of most conventional phylogenies is that species in the Conidae, Turridae and Terebridae should cluster with each other more than with species in the other neogastropod families analyzed, i.e., the Costellaridae, Mitridae and Olividae. Thus, each proposed taxonomy makes clear predictions regarding molecular results. As we show below, the first prediction above is indeed fulfilled by our data. However, the separation of the families of toxoglossate molluscs into a presumably monophyletic superfamily or suborder within the order Neogastropoda is not supported by the data. The results are much more consistent with a "star phylogeny," i.e., all of the neogastropod families analyzed diverged from a common ancestor at approximately the same time.

Preliminary reconstruction of neogastropod phylogeny using mitochondrial ribosomal RNA. Sequences of mitochondrial 16S ribosomal RNA from the neogastropod species, and one mesogastropod species are shown in Table III. Three of the sequences included were part of the >70 sequences published in previous reports on the molecular phylogeny of the genus *Conus* (Monje et al., 1999; Espiritu et al., 2001). The *Conus* species analyzed include *Conus ermineus* (Born, 1778), a piscivorous species from the Atlantic, *Conus textile* ((Linnaeus, 1758), a molluscivorous species collected in the Philippines, and *Conus californicus* (Reeve, 1843), an Eastern Pacific generalist species that probably eats polychaete worms as its major class of prey. Two species conventionally assigned to the subfamily Turrinae were analyzed, *Turris spectabalis* (Reeve, 1843) and *Lophiotoma albina* (Lamarck, 1822). The third turrid analyzed was *Clavus unizonalis* (Lamarck, 1822), in the subfamily Drillinae. In a recent proposal for reclassifying the turrids, the Turrinae and Drillinae were assigned to different families (to be named Turridae and Drillidae (Taylor et al., 1993)). In addition to the turrid and

Table II
Suprageneric Taxonomy According to Vaught (Vaught, 1989)

Species analyzed	Subfamily	Family	Superfamily
Order Mesogastropoda			
Rhinoclavis aspera (Linné, 1758)	Cerithiinae	Cerithiidae	Cerithioidea
Order Neogastropoda			
Oliva miniacea (Röding, 1798)	Olivinae	Olividae	Muricoidea
Mitra mitra (Linné, 1758)	Mitrinae	Mitridae	Muricoidea
Mitra ustulata (Reeve, 1844)	Mitrinae	Mitridae	Muricoidea
Vexillum compressum (Sowerby, 1874)		Costellaridae	Muricoidea
Vexillum granosum (Gmelin, 1791)		Costellaridae	Muricoidea
Conus ermineus (Born, 1778)		Conidae	Conoidea
Conus textile (Linné, 1758)		Conidae	Conoidea
Conus californicus (Reeve, 1844)		Conidae	Conoidea
Turris spectabilis (Reeve, 1843)	Turrinae	Turridae	Conoidea
Lophiotoma albina (Lamarck, 1822)	Turrinae	Turridae	Conoidea
Clavus unizonalis (Lamarck, 1822)	Drillinae	Turridae	Conoidea
Terebra subulata (Linné, 1767)		Terebridae	Conoidea
Terebra crenulata (Linné, 1758)		Terebridae	Conoidea

Table III

	1				50
Conus ermineus	TACGAAGTCG	GA.CCTGCCC	AGTGAG....	TTTTAAACGG	CCGCGGTACT
Conus textile	TACGAAGTCG	GA.CCTGCCC	AGTGAG....	TTTTAAACGG	CCGCGGTACT
Conus californicus	TATGAAGTCG	GA.CCTGCCC	AGTGAA...T	TTTTCAACGG	CCGCGGTACT
Turris spectabilis	TAAGGAGTCG	GA.CCTGCCC	AGTGAA.TTA	TTTTTAACGG	CCGCGGTACT
Lophiotoma albina	TAGGGAGTCG	GA.CCTGCCC	AGTGAA..TT	TTTTTAACGG	CCGCGGTACT
Clavus unizonalis	TAAGGAGTCG	GA.CCTGCCC	AGTGAAG..T	TTTTTAACGG	CCGCGGTACT
Terebra crenulata	TGGGGAGTCG	GA.CCTGCCC	GGTGAAA..T	TTTTTAACGG	CCGCGGTACT
Terebra subulata	ATGGGAGTCG	GA.CCTGCCC	GGTGAA..TT	TTTTTAACGG	CCGCGGTACT
Vexillum compressum	.AAAGAGTCG	GATCCTGCCC	AGTGAAAATT	ATTTTAACGG	CCGCGGTACC
Vexillum granulosum	.AAAGAGTCG	GA.CCTGCCC	AGTGATAA.T	TTTTTAACGG	CCGCGGTACC
Mitra mitra	TAGGGAGTCG	GA.CCTGCCC	AGTGAA....	TTTTCAACGG	CCGCGGTACT
Mitra ustulata	TAGGGAGTCG	GA.CCTGCCC	GGTGAA....	TTTTTAACGG	CCGCGGTACT
Rhinoclavis aspera	TAGGGAGTCG	GA.CCTGCCC	GGTGAAAAA.	.TTTTAACGG	CCGCGGTACT
Oliva miniacea	TGGGGAGTCG	GA.CCTGCCC	GGTGAAAA..	TTTTTAACGG	CCGCGGTACT
	51				100
Conus ermineus	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Conus textile	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Conus californicus	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTGGA
Turris spectabilis	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Lophiotoma albina	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GGAGGCTAGT
Clavus unizonalis	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GGAGGCTAGT
Terebra crenulata	CTGACCGTGC	AAAGGTAGAC	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Terebra subulata	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
Vexillum compressum	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTGTAATT	TAAGGCTAGT
Vexillum granulosum	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTGTAATT	TAAGGCTAGT
Mitra mitra	CTGACCGTGC	AAAGGTAGCA	TAATAATTTG	CCTTATAATT	GAAGGCTGGT
Mitra ustulata	CTGACCGTGC	AAAGGTAGCA	TAATAATTTG	CCTTATAATT	GAAGGCTAGA
Rhinoclavis aspera	CTGACCGTGC	AAAGGTAGCA	TAATCACTTG	CCTTATAATT	GAAGGCTGGT
Oliva miniacea	CTGACCGTGC	AAAGGTAGCA	TAATCATTTG	CCTTATAATT	GAAGGCTAGT
	101				150
Conus ermineus	ATGAATGGTT	TGACAAGAAT	ACACCTGTCT	CTTTTAGGCT	GCCTAGAATT
Conus textile	ATGAATGGTT	TGACAAGAAT	ACACCTGTCT	CTTTTAGATT	ACTTAGAATT
Conus californicus	ATGAATGGTT	TGACAAGAGT	GCAACTGTCT	CTTTATGATT	CAATAGAATT
Turris spectabilis	ATGAATGGTT	TGACAAGAAT	ATGGCTGTCT	CTTTATAATT	TTATAGAACT
Lophiotoma albina	ATGAATGGTT	TGACAAGAAT	ATAGCTGTCT	CTTTATAACT	TTATAGAATT
Clavus unizonalis	ATGAATGGTT	TGACAAGAAT	ATAGCTGTCT	CTTTTTGATT	TATTAGAACT
Terebra crenulata	ATGAATGGTT	TGACAAGAAT	GTAGCTGTCT	CTTCATAATT	TGGTAGAATT
Terebra subulata	ATGAATGGTT	TGACAAGAAT	ATGGCTGTCT	CTTTATAATT	TGATAGAATT
Vexillum compressum	ATGAAAGGTT	TGACAAGAAT	ATAACTGTCT	CCTGTTGGTT	TAATAGAACT
Vexillum granulosum	ATGAAAGGTT	TGACAAGAAT	ATAACTGTCT	CCTTTTGGTT	TAATAGAATT
Mitra mitra	ATGAATGGTT	TGACGAGAAT	AAAGCTGTCT	CTTTGCAACT	TTTTAGAAAT
Mitra ustulata	ATGAATGGTT	TGACGAGAAT	AAAGCTGTCT	CTTTACAACT	CTATAGAAAT
Rhinoclavis aspera	ATGAATGGTT	TGACGAAAGC	ACAGCTGTCT	CTCTCCCGTT	TTATAAAAAT
Oliva miniacea	ATGAATGGTT	TGACGAGAAT	ATTACTGTCT	CTACTTGATT	TACTAGAAAT
	151				200
Conus ermineus	TTATCTTTGG	ATGAAAAAGT	CCAAATATTA	TTAAAAGACA	AGAAGACCCT
Conus textile	TTATCTTTGG	ATGAAAAAGT	CCAGATTTAA	TTAAAAGACA	AGAAGACCCT
Conus californicus	TTATTTTGGG	ATGAAAAAGT	CCTAATATAA	TTAAAAGACA	AGAAGACCCT
Turris spectabilis	TTATTTTAAA	GTGCAGAAGC	TTTAATTAAA	TTAAAAGACA	AGAAGACCCT
Lophiotoma albina	TTATTTTAAA	GTGAAGAAGC	TTTAATGTAA	TTAAAAGACA	AGAAGACCCT
Clavus unizonalis	TTATTTTAAG	GTGAAGAAGC	CTTAATTTAA	TTAAAAGACA	AGAAGACCCT
Terebra crenulata	TTATTTTTAG	GTGAAAAAGC	CTTAATGTAA	TTAATAGACA	AGAAGACCCT
Terebra subulata	TTATTTTCAG	GTGAAAAAGC	CTTGATTAAA	TTAATAGACA	AGAAGACCCT
Vexillum compressum	TTTCTTATAA	GTGAAGAGAC	TTATATACAA	TTAATAGACA	AGAAGACCCT
Vexillum granulosum	TTACTTATAA	GTGAAGAGGC	TTATATACAA	TTAATAGACA	AGAAGACCCT
Mitra mitra	TTATTTTCGG	ATGAAAAAGT	CCGTATACCA	TTAAAAGACA	AGAAGACCCT
Mitra ustulata	TTATTTGCAG	ATGAAAAAGC	CTGCATAATA	TTAAAAGACA	AGAAGACCCT

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Rhinoclavis aspera      TAACATTTGG GTGAAGAGGC CCAAATTGAA TTAAAGGACG AGAAGACCCT
Oliva miniacea          TTATCTTCAG GTGAAGAAGC CTGAATAGAA TTAAAAGACA AGAAGACCCT

                        201                                                250
Conus ermineus          ATCGAGCTTT AGAAAAATTA GTAGAC.TTA A..TATAAAT CAATATAAGT
Conus textile           ATCGAGCTTT AGAGAAGTTA ATAGAC.TTA G..TTTAAAC CAATATAGAT
Conus californicus      ATCGAGCTTG AAATAAATTA ACAAAAGAAA AATTT..... .ATTAG..AC
Turris spectabilis      ATCGAGCTTA AAAAAAATTT TTAGAAACAA ATTAA..... .TAAAA.ATT
Lophiotoma albina       ATCGAGCTTT AAAAAAATCT TTAGAAATTA ATTAA..... .TAAATGATC
Clavus unizonalis       GTCGAGCTTT AAAAAAGTCA ATAGAAATTA CCTTA..... .ATTAA...A
Terebra crenulata       ATCGAGCTTA AAAGAATTTA GTGGATTTTT AAATAG.... .TTTATTGGT
Terebra subulata        ATCGAGCTTG AAGGAATTTG ATGGGTAAAA AATAAGCCTT ATTGGTGGAG
Vexillum compressum     ATCGAGCTTT AAATCAGTTA ATAGAAATAA ATGAAATAAA TG.......C
Vexillum granulosum     ATCGAGCTTG AAATCAATTA ATAGAAATTA AACTGAATAG ACA......T
Mitra mitra             ATCGAGCTTT AAAAAAATTC AGTAGACCAT GTAACTAATT AAA......T
Mitra ustulata          ATCGAGCTTT AAAAGATTTA ATGGGTTAAA AACTTATTAT GAG.....CT
Rhinoclavis aspera      GTCGAGCTTT AGGGGAAGGA GGGAAATTTT ATATTTAT.. ..........
Oliva miniacea          ATCGAGCTTG AAACAAATTA ATGGATTAAA AACAATAAG. .......TAA

                        251                                                300
Conus ermineus          AAAAGGAAAA ..CTACTAAA TACTTTGGTT GGGGCAACCG AGGAGTAAAT
Conus textile           AAAAGGAAAA ..CTATTAAA TACTTTGGTT GGGGCAACCG AGGAGCAAGT
Conus californicus      AAAAAAATTG TGTTGTTAAA CATTTTGGTT GGGGCAACTA AGGAGTAAAA
Turris spectabilis      TTAAAAAATT AGCTAAGAAA TATTTTAGTT GGGGCGACTA AGGAACAAAC
Lophiotoma albina       TTAAAAAATC AGCTATTGAA TATTTTGGTT GGGGCGACTA AGGAACAGAA
Clavus unizonalis       CTAAAAAATC ATCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAAAA
Terebra crenulata       TAAGAGGTAT AACTGCTGAG ATTTTTGGTT GGGGCGACTG AGGAACATTT
Terebra subulata        AAAAGGGGTA AACTGTTGAA TATTTTGGTT GGGGCGACTG AGGAACAGGA
Vexillum compressum     ATAAAATCAA ATCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAGCT
Vexillum granulosum     ATAAAACTAA GTCTATTAAA AATTTTGGTT GGGGCGACTA AGGAACAGCT
Mitra mitra             CTAGAAAGTC ATCTCCTGAA AATTTTAGTT GGGGCGACTA AGGAACAAAT
Mitra ustulata          TAAGACAGTT ATCCATTAAG AATTTTAGTT GGGGCGACTA AGGAACAAAC
Rhinoclavis aspera      .AAATACATG GCCTCACTCA CCCTTTAGTT GGGGCGACTA GGGAACAATA
Oliva miniacea          TAAAAGTGTT AGCCATTGAA CATTTTGGTT GGGGCGACTA AGGAACAAAC

                        301                                                350
Conus ermineus          AGAGCCTCCT T.....TGAA TTACAAAT.C CTA.CATGTA ATTGATCC.A
Conus textile           AAAGCCTCCT TT.....AAA TA.GTAAATC TTG.CTTGTG .TTGATCC.A
Conus californicus      AAAGCCTCCT T...TATGTT AAGATAAA.C TAA.CAAGTA C.TGATCC.A
Turris spectabilis      AAAGCTTCCT T...TG.ATA AA.AATAA.C ATTT.AAGTA .TGGATCC.A
Lophiotoma albina       AAAGCTTCCT T...AA.ATA ATTAATGA.C ATT.CATGTA .TTGATCC.A
Clavus unizonalis       AGAGCTTCCT T...T.TACA TAATAAAATC TAT..AAATT .TTGATCC.A
Terebra crenulata       AAAGCTTCCT T...TA.ATG TAGATATA.C GTA.CAAGTG .TTGATCC.A
Terebra subulata        AAAGCTTCCT T...TG.TGG T.GTGATA.C ATA.CAGGTG .TTGATCC.A
Vexillum compressum     AAAGCTTCCT ATTAAAA... ..TTTAAATC TTTTCAAGTA G.TGA.CCCA
Vexillum granulosum     AAAGCTTCCT ATTTAA.... ..TTTAAA.C CTT.CAAGTA G.TGATCC.A
Mitra mitra             AAAGCTTCTT TAAAACACGT CTTTTG...C TCATTAGCTT ..TGATCC.A
Mitra ustulata          AAAGCTTCCT CATTAGCA.. TTTTTA..CC T.AT.AAGTT C.CGATCC.A
Rhinoclavis aspera      AAAGCTTCCC TT......AT TTTCTAAA.T TTATATATTA ..GGATCC.A
Oliva miniacea          AAAGCTTCCC T.....ATAT AAAATAGGTT CACCGACAT. ..TGATCC.A

                        351                                                400
Conus ermineus          AAAA...TTT TGATCAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Conus textile           AAA...TTTT TGATCAAGGG AA...TTACT TACC.GTAGG GATAACAGCA
Conus californicus      AAC...TTTT TGATCAAAGA AAA...TAGT TACC.GTAGG GATAACAGCA
Turris spectabilis      GAA...TGTT TGATTGAGAG AA...TTAGT TACC.GTAGG GATAACAGCA
Lophiotoma albina       AAA...TTTT TGATTAAAGA ATA...TAGT TACC.GTAGG GATAACAGCA
Clavus unizonalis       AAAA..TTTT TGATTAAAAG AA...TTAGT TACC.GCAGG GATAACAGCA
Terebra crenulata       AAA...TTAA TGATTAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Terebra subulata        AAAG...TGT TGATTAAAGG AA...TTAGT TACC.GTAGG GATAACAGCA
Vexillum compressum     GAAAA..TTC TGGTTAAAGA AA...TTAGT TACC.GTAGG GATAACAGCA
Vexillum granulosum     GAAAA..TTC TGATTAAAGA AA...TTAGT TACC.GTAGG GATAACAGCA
Mitra mitra             GAAA...TTC TGATTAATAA AA...TTAGT TACC.GTAGG GATAACAGCA
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Mitra ustulata	GAAGCA.TTC	TGATTAATAG	AA...TTAGT	TACC.GTAGG	GATAACAGCA
Rhinoclavis aspera	GCATTAAAGC	TGCTGATCAA	AAGAATTAGT	TACCCGCAGG	GATAACAGCA
Oliva miniacea	AAAGA..TTT	TGATTAATGA	AA...TTAGT	TACC.GTAGG	GATAACAGCA

	401				450
Conus ermineus	TTATCTTTTT	CAAGAGCCCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Conus textile	TTATCTTTTT	TAAGAGCCCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Conus californicus	TTATCTTTTT	TGAGAGTTCC	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Turris spectabilis	TTATCTTTTT	TGAGAGTTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Lophiotoma albina	TTATCTTTTT	TGAGAGTTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Clavus unizonalis	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Terebra crenulata	TTATCTCCTT	TGAGAGTTCT	TATCGAAAAG	GGGGTTTGTG	ACCTCGATGT
Terebra subulata	TTATCCTTTT	TGAGAGTTCA	TATCGAAAAA	GGGGTTTGTG	ACCTCGATGT
Vexillum compressum	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Vexillum granulosum	TTATCTTTTT	TGAGAGCTCA	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Mitra mitra	TTATCTTTTT	TGAGAGCTCT	TATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Mitra ustulata	TTATCTTTTT	TGAGAGTTCT	CATCGAAAAA	AAGGTTTGTG	ACCTCGATGT
Rhinoclavis aspera	TTATCCTTCT	TGAGAGACCA	TATCGAAAGA	AGGGGTTGTG	ACCTCGATGT
Oliva miniacea	TTATCTTTTT	TGAGAGCTCT	AATCGAAAAA	AAGGTTTGTG	ACCTCGATGT

	451				500
Conus ermineus	TGGACCAGAA	TATCCTGAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Conus textile	TGGACCAGAA	TGTCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Conus californicus	TGGACCAGAA	TATCCTGAAG	ATGCAGAAGT	CTTCAAGGG.	...TTGGTCT
Turris spectabilis	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Lophiotoma albina	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Clavus unizonalis	TGGACCAGAA	TATCCTAAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Terebra crenulata	TGGACCAGAA	TGTCCTGAAG	ATGCAGAAGT	CTTTAAGGG.	...TTGGTCT
Terebra subulata	TGGACCAGAA	TGTCCTGAAG	ATGCAGAAGT	CTTCAAGGG.	...TTGGTCT
Vexillum compressum	TGGACCAGAA	TATCCCAAAG	ATGTAGCAGT	CTTTAAGGGA	GGGTTGGTCT
Vexillum granulosum	TGGACCAGAA	TATCCTAAAG	ATGCAGCAGT	CTTTAAAGG.	...TTGGTCT
Mitra mitra	TGGACCAGAA	TATCCTAAAG	ATGCAGCAGT	CTTTAAGGG.	...TTGGTCT
Mitra ustulata	TGGACCAGAA	TGTCCTAAAG	ATGCAGCAGT	CTTTAAGGG.	...TTGGTCT
Rhinoclavis aspera	TGGACTAGGA	TATCCGGATG	GTGCAGAAGC	CCTCAAAGG.	...TTGGTCT
Oliva miniacea	TGGACCAGAA	TATCCCAAAG	GTGTAGCAGC	CTTTAAAGG.	...TTGGTCT

	501	511
Conus ermineus	GTTCGACCAT	T
Conus textile	GTTCGACCAT	T
Conus californicus	GTTCGACCAT	T
Turris spectabilis	GTTCGACCAT	T
Lophiotoma albina	GTTCGACCAT	T
Clavus unizonalis	GTTCGACCAT	T
Terebra crenulata	GTTCGACCAT	T
Terebra subulata	GTTCGACCAT	T
Vexillum compressum	GCTCGACCAT	T
Vexillum granulosum	GTTCGACCAT	T
Mitra mitra	GTTCGACCAT	T
Mitra ustulata	GTTCGACCAT	T
Rhinoclavis aspera	GTTCGACCAT	T
Oliva miniacea	GTTCGACCAT	T

Table III. The DNA sequences above were obtained from tissue collected directly from live gastropods. The live specimen was cooled down in an ice bath for 5-10 min, the shell was smashed with a mallet, and the specimen quickly dissected on an ice block. The fresh hepatopancreas of the dissected snail was either quickly placed in liquid nitrogen or immediately extracted with buffer. The method used for DNA extraction is basically the rapid one-step extraction (ROSE) method of Steiner et al. (Steiner et al., 1995). This technique eliminates the need for organic solvent extraction and enzyme digestion, and involves a rapid one-step process. The DNA extracted was analyzed by agarose gel-electrophoresis, and high molecular weight (>25kb) DNA was routinely obtained by these procedures. The initial extraction gave a 260:280 ratio that was considerably less than that for pure DNA (circa 1.7). Most samples were further purified using centrifugal dialysis (Milipore), concentrating the DNA (to ~7mg/ml) and removing lower molecular weight impurities. Thus, most samples analyzed had an A260:A280 ratio greater than 1.6. After one year of storage, agarose gel-electrophoresis suggested that the molecular weight of the DNA remained >30kb. The primers used for PCR as described in Monje et al. (Monje et al., 1999). All sequences above have been deposited in Genbank.

Conus species analyzed, two other Conoidean species in the genus *Terebra* (*T. crenulata* (Linnaeus, 1758) and *T. subulata*(Linnaeus, 1767)) are included in this survey. *Terebra subulata* is a venomous species, while *T. crenulata* is one of the larger *Terebra* species that do not have a venom duct.

The five other species from which mt 16S rRNA sequences were obtained include *Mitra mitra* (Linnaeus, 1758) and *Mitra ustulata* (Reeve, 1844) (in the Mitridae), *Vexillum compressum* (Sowerby, 1874) and *Vexillum granosum* (Gmelin, 1791) (in the Costellaridae) and *Oliva miniacea* (Röding, 1798) (in the Olividae). Both *Mitra* and *Vexillum* were originally included in the Mitridae. However, on the basis of differences in the radula, the Costellaridae have been recognized as a separate family in more recent taxonomic work.

The relevant mitochondrial sequences for the 14 species are shown in Table III, and these were aligned as described in the Table legend. A phylogenetic reconstruction was made using either parsimony or maximum distance (see Fig. 2). In addition, the divergence was quantitated using the Kimura two-parameter method; the pairwise divergence values for all species analyzed is shown in Table IV.

If we use the rate previously used for *Conus* of 0.33% per 10^6 years (range 0.24-0.40%), which was calibrated on the basis of the fossil record of the genus (Kohn, 1990), the time of divergence of the various species within each family can be estimated. Thus, some of the species appear to have diverged in the Miocene, including species in the same genus (i.e., *Vexillum compressum* and *Vexillum granosum* in the Costellaridae), as well as some species assigned to different genera (i.e., *Turris spectabilis* and *Lophiotoma albina* – however, these are both assigned to the same subfamily, the Turrinae). In contrast, some species in the same genus appear to have diverged significantly earlier, in the Eocene. Such early diverging taxa include *Terebra subulata* and *Terebra crenulata* (Terebridae) and *Mitra mitra* and *Mitra ustulata* (Mitridae).

The data generally support the conventional assignment of the species in Table II into the family groups indicated. The three species of Turridae, for example, exhibit a divergence range (7.2 - 11.3%) which is clearly smaller than their divergence from other neogastropods (14.4 - 20.8%) or from the mesogastropod outgroup species (29.1 - 30.1%).

Implications of the molecular data. The results described above, though preliminary, support some rather unconventional phylogenetic hypotheses regarding the neogastropod families analyzed. We summarize the major trends indicated by the data, and discuss each in turn:

1) All neogastropod groups included in this study are approximately equally divergent from the mesogastropod species used as the outgroup (the cerithid *Rhinoclavis aspera*).

2) In general, all neogastropod groups (which can be assigned to six different families by conventional taxonomy) are approximately equidistant from each other, with the pairwise divergences between neogastropod families being less than the divergence from the mesogastropod *Rhinoclavis*.

3) The Turridae exhibit an apparently smaller divergence distance from all other neogastropod groups.

4) The Costellaridae appear to be closer to the Turridae than to any other neogastropod group (and vice versa).

The % divergence from the mesogastropod *Rhinoclavis aspera* is approximately equal for all neogastropod groups analyzed. If we use the values for the rate of divergence within the genus *Conus* (derived from the analysis of seventy different *Conus* species and correlating the divergence distance values with the fossil record), the age of the last common ancestor between *Rhinoclavis* and the neogastropod families included in this study is

TABLE IV. Kimura Two - Parameter Divergence Distances (%)

	Conidae			Turridae			Terebridae		Costellaridae		Mitridae		Olividae	Mesogastropod
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Conus ermineus*	0.0	7.31	15.3	20.8	18.3	17.0	21.9	24.0	23.8	19.9	21.4	22.5	23.3	29.5
2. *Conus textile*		0.0	15.2	20.8	19.7	15.6	20.7	20.8	22.0	19.4	21.7	21.6	22.5	29.3
3. *Conus californicus*			0.0	18.9	17.1	16.3	20.3	19.3	21.7	19.0	20.1	19.6	19.1	29.2
4. *Turris spectabilis*				0.0	7.2	11.3	19.3	17.5	17.0	16.7	18.0	15.9	18.0	29.1
5. *Lophiotoma albina*					0.0	10.4	17.3	16.0	14.9	14.4	17.2	17.3	17.0	30.1
6. *Clavus unizonalis*						0.0	18.0	17.9	15.1	14.9	17.2	16.7	16.4	28.6
7. *Terebra crenulata*							0.0	12.7	22.5	20.4	23.6	18.1	19.6	31.4
8. *Terebra subulata*								0.0	22.6	20.9	22.7	18.1	18.7	31.4
9. *Vexillum compressum*									0.0	6.5	22.0	22.2	19.6	32.7
10. *Vexillum granulosum*										0.0	21.7	20.2	19.9	29.1
11. *Mitra mitra*											0.0	14.3	19.9	28.3
12. *Mitra ustulata*												0.0	17.1	29.6
13. *Oliva miniacea*													0.0	29.4
14. *Rhinoclavis aspera*														0.0
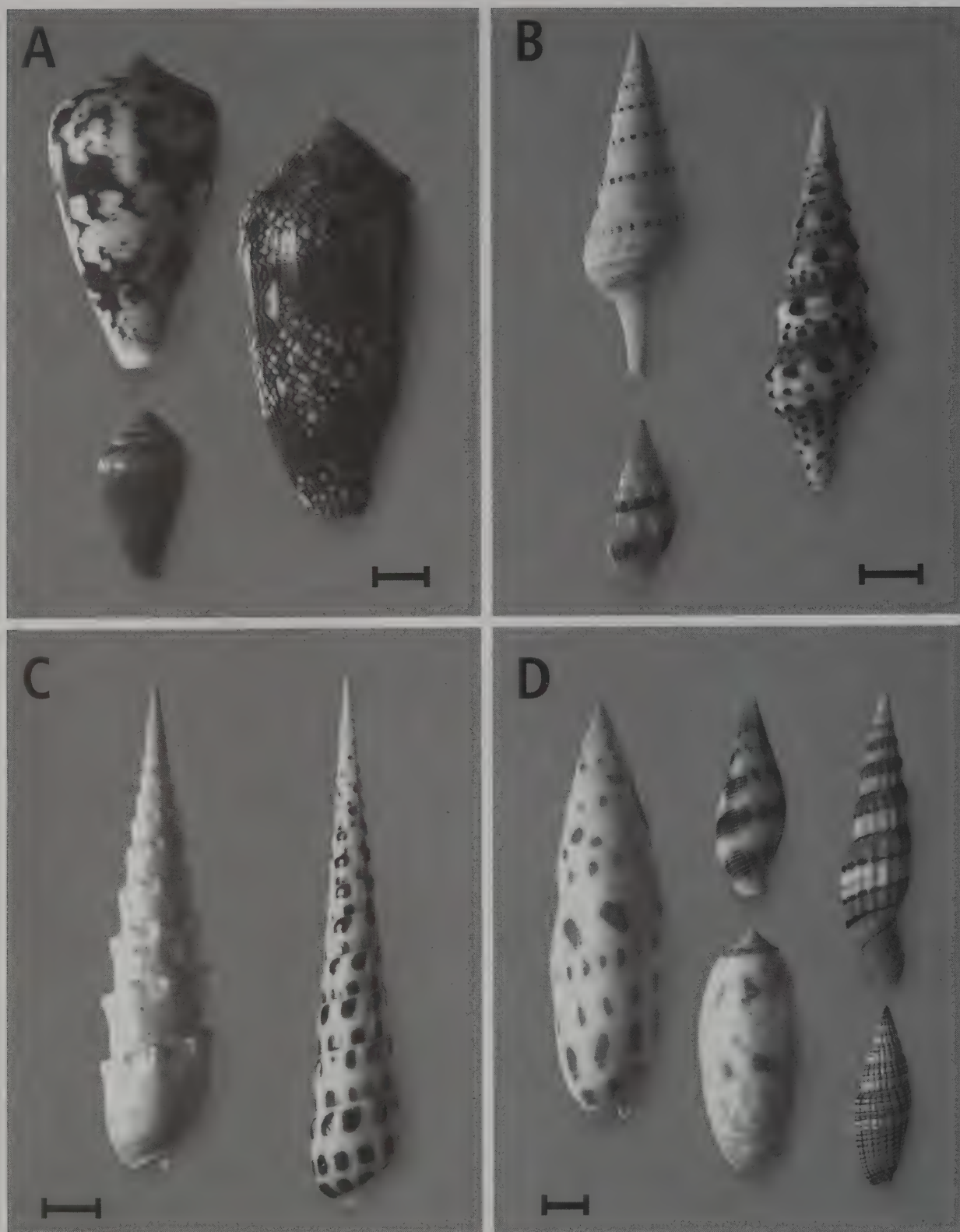
Figure 1. Neogastropod families analyzed in Tables III and IV: Conidae (A); Turridae (B); Terebridae (C) and three non-Conoidean families (D), Mitridae, Costellaridae and Olividae. The *Conus* species from top left, clockwise, are *Conus ermineus* (Bonaire), *Conus textile* (Philippines), *Conus californicus* (California, USA). Turridae analyzed, from top left, clockwise: *Lophiotoma albina* (Philippines), *Turris spectabilis* (Philippines), *Clavis unizonalis* (Philippines). The *Terebra* species analyzed: left, *Terebra crenulata* (Western Samoa); right, *Terebra subulata* (Australia). Other neogastropods not belonging to the superfamily Conoidea: from top left, clockwise, family Mitridae - *Mitra mitra* (Philippines), *Mitra ustulata* (Philippines); family Costellaridae - *Vexillum compressum* (Philippines); *Vexillum granosum* (Philippines), family Olividae - *Oliva miniacea* (Philippines). Color figure prepared by Kerry Matz.

The localities indicated above are of the actual specimens figured. For specimens actually discussed and mit.DNA analyzed, all were from the Philippines except for *Conus ermineus* and *Conus californicus*, which were from Bonaire and California, respectively.

estimated at ca. 84-100 mya. Whether the rate-of-divergence parameter can be extrapolated linearly to that extent is one reservation in this calculation.

The other major result from this study is that all six neogastropod families are essentially equidistant from each other with the exceptions noted below. If one applies the calculation of age of divergence of *Conus* from the other five neogastropod families, the best estimate is that this divergence of neogastropod families occurred close to the K-T boundary, during the late Cretaceous or the Paleocene. The data therefore strongly suggest that a single ancestral line diverged from the mesogastropod ancestor sometime during the Mesozoic, and gave rise to the six neogastropod families in this study sometime around the K-T boundary.

An anomaly in the data is that the divergence distance of the turrids from all other neogastropod groups is consistently less than calculated for any other pair of families. It should be noted that the Turridae are generally deeper water molluscs than the other groups analyzed, with some very deep-water forms. We observed in the previous study of *Conus* that there was a similar anomaly in calculating the divergence of the fish-hunting *Conus* species from mollusc-hunting *Conus* using *Conus textile*, a shallow water mollusc-hunting species, vs. *Conus gloriamaris*, which typically lives at depths of 100 meters. Whether a deep-water habitat (with lower temperatures and perhaps longer generation times) can account for the apparently less divergence seen between the Turridae and other neogastropod groups remains to be established. Other explanations for these data cannot be eliminated at this time.

The most surprising result was the lack of evidence for clustering of toxoglossate families, conventionally included in the superfamily Conoidea (Conacea, Toxoglossa). Thus, the Turridae, Conidae and Terebridae appear to be no more closely related to each other than they are to any of the other neogastropod families. Indeed, among the groups analyzed, the closest relationship between families appears to be between the families Costellaridae and Turridae. The molecular results raise the issue of whether the toxoglossate molluscs are a monophyletic group; a previous analysis also failed to group *Conus* and *Hastula* (in the Terebridae) together as a clade (Harasewych et al., 1997). The species in the Turridae analyzed appear to be less diverged from the two species in the Costellaridae than they are from *Conus* and *Terebra*.

Additionally, the two Costellarid species are significantly more distant from the Mitridae than from the Turridae, which provides strong molecular support for the separation of Costellaridae from Mitridae into distinct families. These two groups do not appear more closely related to each other than any other pair of neogastropod families analyzed.

If the Costellaridae and Turridae are indeed the most closely related families, the separation of Turridae, Terebridae and Conidae into a superfamily division separated from other neogastropod groups would not be tenable. Although the results are admittedly limited both with respect to the number of species analyzed and the number of genetic loci measured, they raise fundamental questions about the conventional taxonomic scheme presently used for Neogastropoda.

The neogastropod families included in this study seem like a classical star phylogeny. In many ways, the data have striking parallels in the evolution of mammalian orders. The molecular analysis of mammals shows a similar sudden diversification near the K-T boundary. It is tempting to hypothesize a common cause for these similar patterns: the geological catastrophe that led to the Cretaceous extinction. The parallel can be extended: just as the complete extinction of the dinosaurs on land provided an opportunity for the mammalian radiation, the total extinction of ammonites in marine habitats may have given a once in 10^8 year ecological opportunity for predatory gastropod lineages to undergo an unprecedented radiation.

IV. Discussion and Perspectives

Since the first biochemical study of *Conus* venoms, considerable progress has been made in understanding the molecular mechanisms underlying snail envenomation. It is clear that the success of the cone snails has been in large part due to the evolution of a remarkable array of conotoxins, as the pharmacological agents underlying the activity of their venoms. It is estimated that there are ca. 50,000 different molecular forms of conotoxins in the venoms of living cone snails. At the genetic level, this has involved an unprecedented diversification of a few gene superfamilies. It appears that the cone snails' success is due in part to the ability to mutate these genes as changes in the environment occur over a geological time period. What this mechanism of hypermutation is remains to be elucidated, but in effect, as an aggregate, the genus *Conus* has apparently evolved appropriate new conotoxins to meet the challenges of new ecological situations during the entire Tertiary period. The extraordinary pharmaceutical properties of *Conus* venom peptides makes them useful as basic tools in neuroscience, as diagnostic agents, and somewhat unexpectedly, as therapeutic drugs.

In the results presented above, we provide an indirect assessment of whether other groups included in the superfamily Conoidea might have underlying strategies of envenomation using conotoxin-like peptides, as has been established for *Conus*. The analysis of the pedigree of various neogastropod families discussed in the sections above suggest that instead of having various stem groups within Conoidea from which the cone snails evolved, the phylogeny fits a star phylogeny better than a branching tree phylogeny. This implies that around the K-T boundary, there was a radiation of all of the different neogastropod groups at approximately the same time. The predicted phylogenetic reconstruction, if confirmed, appears to us to make it less likely that groups such as the Turrinae, and the Drillinae within the family Turridae, or the venomous Terebridae (such as *Terebra subulata*) will overlap considerably with the molecular

and genetic strategy of the cone snails. The possibility that all of the major toxoglossate groups (Conidae, Turridae and Terebridae) arose at the same time as nonvenomous families in Neogastropoda (Mitridae, Olividae) increases the probability that the different Conoidean groups may each have evolved its own characteristic venom components. The probability that different genes may have been recruited for use in venom in the course of their divergence from a common ancestral form is increased by our results. A branching tree organization of Conoidean families would have been consistent with a stepwise evolution of venom genes. This becomes a less tenable alternative if all the neogastropod families diverged from each other at more or less the same time, as suggested by a star phylogeny. Clearly, the only way to settle this question definitively is to undertake the direct analysis of turrid and Terebrid venoms.

Finally, the molecular analysis presented above suggests that the standard taxonomic scheme for neogastropod phylogeny needs reevaluation.

A

Conus ermineus
Conus textile
Conus californicus
Turris spectabilis
Lophiotoma albina
Clavus unizonalis
Vexillum compressum
Vexillum granulosum
Terebra crenulata
Terebra subulata
Mitra mitra
Mitra ustulata
Oliva miniacea
Rhinoclavis aspera

0.10
substitutions per 100 residues

B

100
Conus ermineus
74
Conus textile
Conus californicus
93
Turris spectabilis
Lophiotoma albina
Clavus unizonalis
99
Terebra crenulata
Terebra subulata
100
Vexillum compressum
Vexillum granulosum
73
Mitra mitra
Mitra ustulata
Rhinoclavis aspera
Oliva miniacea

100.00
substitutions per 100 residues

Figure 2. Two phylogenetic reconstructions of several gastropod families. The mitochondrial 16S ribosomal RNA sequence data were obtained as described in the legend to Table III. The sequence alignment shown in Table III was used to generate the phylogenetic trees. (A) A phylogenetic reconstruction using a heuristic search with a minimum evolution distance criterion. In this reconstruction, uncorrected distance parameters were calculated and used to search for optimal trees. (B) An alternative phylogenetic reconstruction using a heuristic search with parsimony as an optimality criterion. A bootstrap analysis was performed to assign confidence levels to groupings in the tree. Confidence levels are shown on each branch. Groupings with levels below 50% are not shown.

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Morphological prerequisites for understanding neogastropod phylogeny

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KEY WORDS: Neogastropoda, phylogeny, sister group, anatomy, foregut, feeding mechanisms

ABSTRACT Analysis of anatomy confirms that the neogastropods are a clade, for which monophyly is supported by several apomorphies and common characters, nearly all of which confined to the anterior foregut. The most primitive neogastropods are characterised by short proboscis with the basal buccal mass and with odontophoral and radular muscles passing through the nerve ring and joining the columellar muscle. None of the existing theories on neogastropod evolution (Amaudrut, 1898; Ponder, 1974; Golikov & Starobogatov, 1988; Ponder & Lindberg, 1997; Riedel, 2000) adequately describes the origin and radiation of the group. The potential sister group of Neogastropoda should be found among carnivorous groups of Sorbeoconcha with underived foregut. The Tonnoidea are unlikely a sister group of the neogastropods.

RIASSUNTO L'analisi dei dati anatomici disponibili conferma che i neogasteropodi sono un clado, la cui monofilia è supportata da diverse apomorfie e caratteri comuni, principalmente riguardanti la porzione anteriore del canale digerente.
I neogasteropodi più primitivi sono caratterizzati da una proboscide corta con la massa boccale basale e con i muscoli odontoforale e radulare uniti al muscolo columellare e passanti attraverso l'anello nervoso.
Nessuna delle teorie sinora proposte per l'evoluzione dei neogasteropodi (Amaudrut, 1898; Ponder, 1974; Golikov & Starobogatov, 1988; Ponder & Lindberg, 1997; Riedel, 2000) descrivono adeguatamente l'origine e la radiazione del gruppo. Il potenziale sister-group dei Neogastropoda dovrebbe essere ricercato tra i gruppi carnivori dei Sorbeoconcha con un apparato alimentare anteriore relativamente non modificato. I Tonnoidea sono probabilmente poco plausibili come sister-group dei neogasteropodi.

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INTRODUCTION

The past decade witnessed significant progress in studies of the Neogastropoda, including the anatomy of various groups, ultrastructure, ontogeny and molecular phylogeny. Among most important, can be mentioned the publications of BALL *et al.* (1997, 1997a) on the ontogeny of different structures of the digestive system in the postveliger stages of *Nucella*, the publication of HARASEWYCH *et al.* (1997) on the molecular phylogeny of neogastropods, the series of studies of different groups of Conoidea (KANTOR & TAYLOR, 1991; TAYLOR, KANTOR & SYSOEV, 1993) and many others. Nevertheless, the phylogenetic hypotheses of the radiation of the Neogastropoda as well as the relationships with other prosobranchs are still the subject of different speculations.
Although there are few published comparable phylogenetic schemes for Neogastropoda, we can see major differences in these recently proposed hypotheses of neogastropod evolution, namely those of KANTOR (1996), PONDER & LINDBERG (1997) and RIEDEL (2000) (Figure 1). There are major differences in opinions on the position of most families, as well as in the treatment of characters and character states.

The differing opinions on the neogastropod evolution are likely the result of high rate of homoplasy within the neogastropods. Numerous lineages (20+ families) proliferated rapidly during the Cretaceous, with tendencies to modify organ systems in parallel fashions in many of them (HARASEWYCH *et al.*, 1997). As a consequence, relatively few morphological characters originating during the initial radiation can be identified. Therefore, cladistic analysis of the families and major groupings relationships is often unsatisfactory, with very poor resolution (eg. KANTOR, 1996).
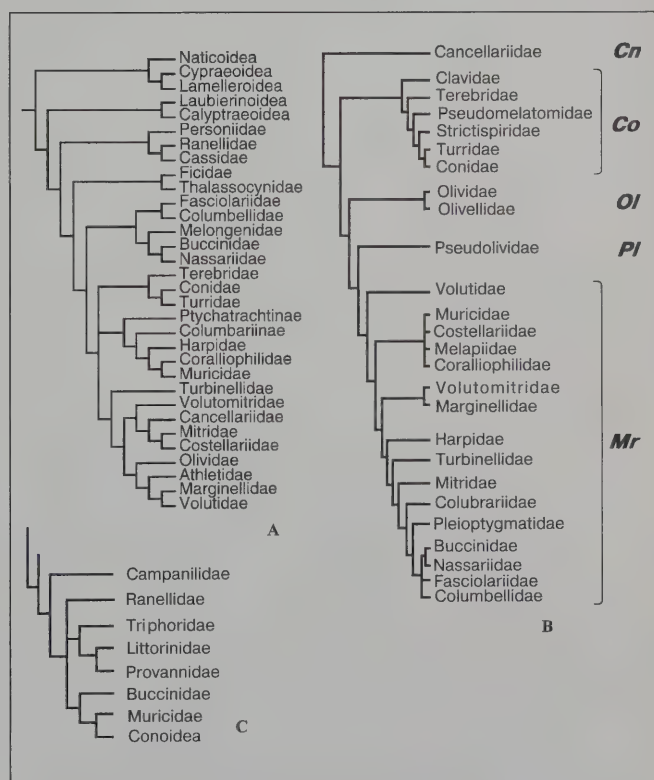
Figure 1. Phylogenetic hypothesis of the Neogastropod relationships. A — after RIEDEL (2000) (combined by M.G.Harasewych). B — after KANTOR (1996). *CN* — suborder Cancellarioidei, *CO* — suborder Conoidei, *OL* — suborder Olivelloidei, *PL* — suborder Pseudolivoidei, *MR* — suborder Muricoidei. C — after Ponder & Lindberg (1997) (modified).
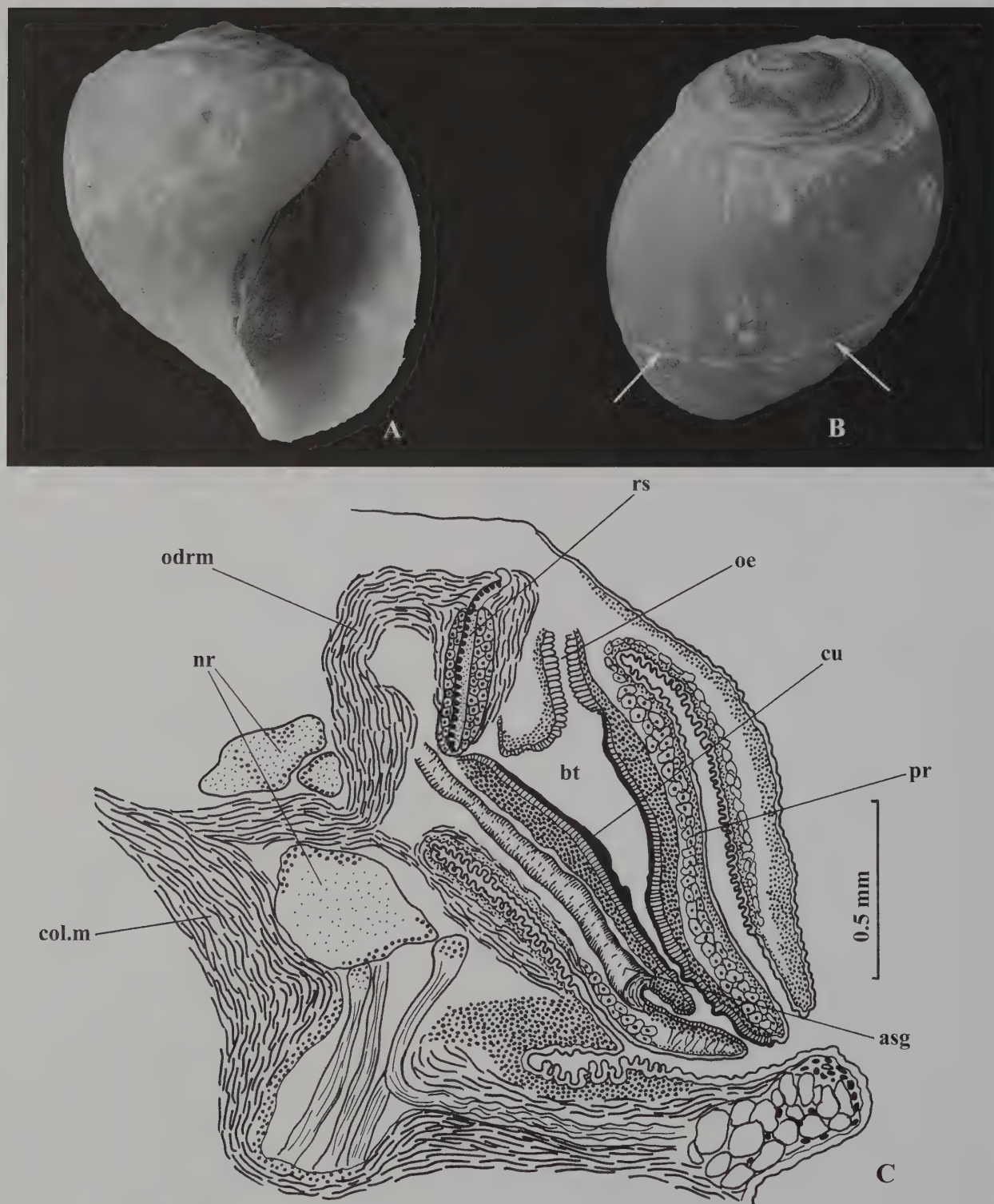
Figure 2. *Benthobia tryoni* Dall, 1889 (Pseudolividae). Eastern Atlantic, 19°45'N, 18°21'E, 2850 m [after KANTOR, 1991]. A, B — apertural and dorso-lateral views of the shell, shell length 8.6 mm. Arrows on B shows the spiral sulcus. C — semidiagrammatic longitudinal section through the anterior foregut. Abbreviations: asg — accessory salivary gland; bt — buccal tube; col.m — columellar muscle; cu — cuticular lining of the buccal tube; nr — nerve ring; odrm — odontophoral retractor; oe — oesophagus; pr — proboscis; rs — radular sac.

Finally, there are certain families of Neogastropoda (although sometimes not generally accepted), which hardly can be defined in cladistic terms, since they lack autapomorphies, e.g. family Pseudolividae. This family is still often considered as subfamily of Olividae (e.g. SMITH, 1998), but in my opinion definitely deserves familial status and is not closely related to any Olividae (KANTOR, 1991). This family is readily distinguished by a combination of some anatomical characters — short proboscis with the buccal mass situated at its base, the passage of the odontophoral retractors through the nerve ring, the presence of an accessory salivary gland (Figure 2C) together with the presence of the spiral sulcus on the body whorl (Figure 2 B, arrows indicate the spiral sulcus). None of these characters are autapomorphies of Pseudolividae and are found in other neogastropods. For example the spiral sulcus is also present in genus *Ceratoxancus* Kuroda, 1952 (subfamily Ptychatractinae, Turbinellidae) (KANTOR & BOUCHET, 1997).

The molecular phylogeny encounters the similar problems. Thus, the analysis of the 18s rDNA cannot resolve the Neogastropoda as a clade, since the rapid adaptive radiation of the neogastropods is probably below the limits of resolution even of the entire gene sequence (HARASEWYCH *et al.,* 1997). On the contrary, the cytochrome *c* oxidase I seem to be more suitable for resolution within the neogastropods, but not very useful for general analysis of the higher Caenogastropoda, thus leaving open the question of the sister group.

The main purpose of this publication is to summarise existing hypotheses on neogastropod radiation and to show the possible sequence of morphological transformations of the digestive system. The latter may allow the definition of plesiomorphic states for some characters and thus may give a clue for determining the potential sister groups of the Neogastropoda, which should be examined more carefully.

SYNAPOMORPHIES OF THE NEOGASTROPODA

TAYLOR and MORRIS (1988) summarised and provided an analysis of the main apomorphies of the neogastropods. They listed the following characters (below I will provide the brief morphological overview of these characters):

1) Two pairs of salivary glands.

Both primary and accessory salivary glands have rather variable morphology.

Primary salivary glands are in most cases acinous, but may be tubular in some Conoidea, such as Mangeliinae and Raphitominae (family Conidae — in this publication the classification of Conoidea, proposed by TAYLOR *et. al.,* 1993 is used) (TAYLOR *et. al.,* 1993; KANTOR & TAYLOR, this volume) and few species of Crassispirinae (Turridae) (KANTOR, MEDINSKAYA & TAYLOR, 1997). Primary salivary glands are absent in some Conoidea, in which the venom and radular apparatus are absent (TAYLOR *et al.*, 1993; TAYLOR, 1990). In most cases the glands are paired. In some Buccinidae and Buccinulidae the glands are fused without a visible border, but still retain the paired ducts, e.g. *Habevolutopsius* (KANTOR, 1990), *Chlanidota* (HARASEWYCH & KANTOR, 1999). The ducts of the glands (when present) enter the buccal cavity near the entrance of the radular diverticulum into the buccal cavity and are always lined with ciliated epithelium.

In radular-less Coralliophilidae the initially paired ducts fuse together and the unified duct passes dorsally to the anterior oesophagus towards its opening into the buccal tube (WARD, 1965; KANTOR, 1995). In some groups, e.g. all Conoidea and Cancellariidae the ducts are short, free along their length (TAYLOR *et. al.,* 1993; GRAHAM, 1966). On the contrary, in the majority of Muricoidea (sensu PONDER, 1974) the ducts, after leaving the glands soon become "embedded" into the walls of the anterior oesophagus (usually just in front of the valve of Leiblein), which they follow towards their opening. In Buccinoidea (which usually possess long or very long proboscis) there is an intermediate situation — the ducts are free along most of their length (although usually are attached to the oesophagus by numerous connective tissue fibres) and enter the walls of the anterior oesophagus close to their opening.

Finally, in Mitridae the ducts the enter epiproboscis and open at its tip (PONDER, 1972). The epiproboscis (an autapomorphy of the family) is a muscular rod, situated in its own sheath and able to protrude through the mouth opening. In at least one species of Mitridae (a still unnamed species of *Eumitra*, unpublished), possessing an epiproboscis, the ducts open in their typical position.

The situation with accessory salivary glands is not less complicated. Accessory glands are paired tubular organs (single in some species) with ducts that fuse together into the single duct, which then passes ventrally to the oesophagus and opens into anteriormost part of the buccal tube. The ducts of accessory salivary glands are always lined with non-ciliated epithelium. The gland is formed by two epithelial layers, separated by thin layer of circular muscle fibres.

In *Benthobia* (Pseudolividae) the gland has a normal histology, but possesses a very large muscular bulb with a broad lumen at the proximal end (KANTOR, 1991). It is possible that this bulb functions as a propulsive organ propelling the secretion of the gland through the mouth opening.

Finally a most unusual gland is found in *Persicula* (Marginellidae). COVERT & COVERT (1995) called them acinous accessory salivary glands. The gland is large, composed of two
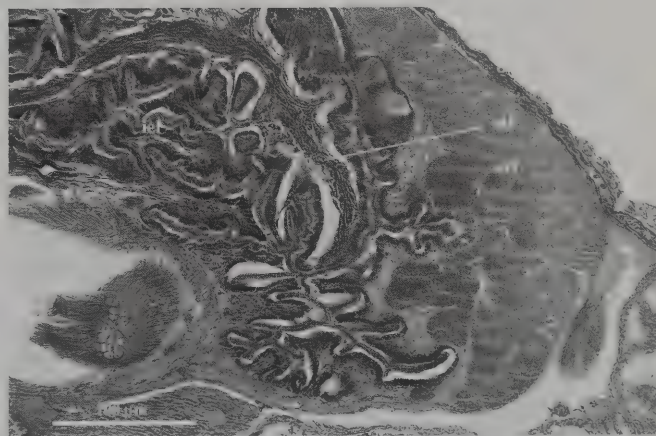
Figure 3. Longitudinal section through the posterior part of the accessory salivary gland of *Persicula persicula* (L., 1758) (Marginellidae). Abbreviations: iel — layer of internal epithelium; ml — layer of muscle fibers; oel — outer epithelial layer

histologically very distinct layers of tissue (Figure 3). The outer layer is of large oval cells with a granulated cytoplasm. The inner one is formed of strongly staining epithelium forming numerous partitions, and underlined by the layer of muscle fibres. The duct of this gland is lined with non-ciliated epithelium and opens into the buccal tube, as in other neogastropods.

2) Dorsal midgut gland.

The midgut gland is most often called the gland of Leiblein in Muricoidea, and venom gland in Conoidea. The homology of both structures, as well as the transformation of the gland of Leiblein and associated with it glandular dorsal folds of mid-oesophagus are discussed in details by PONDER (1970, 1974).
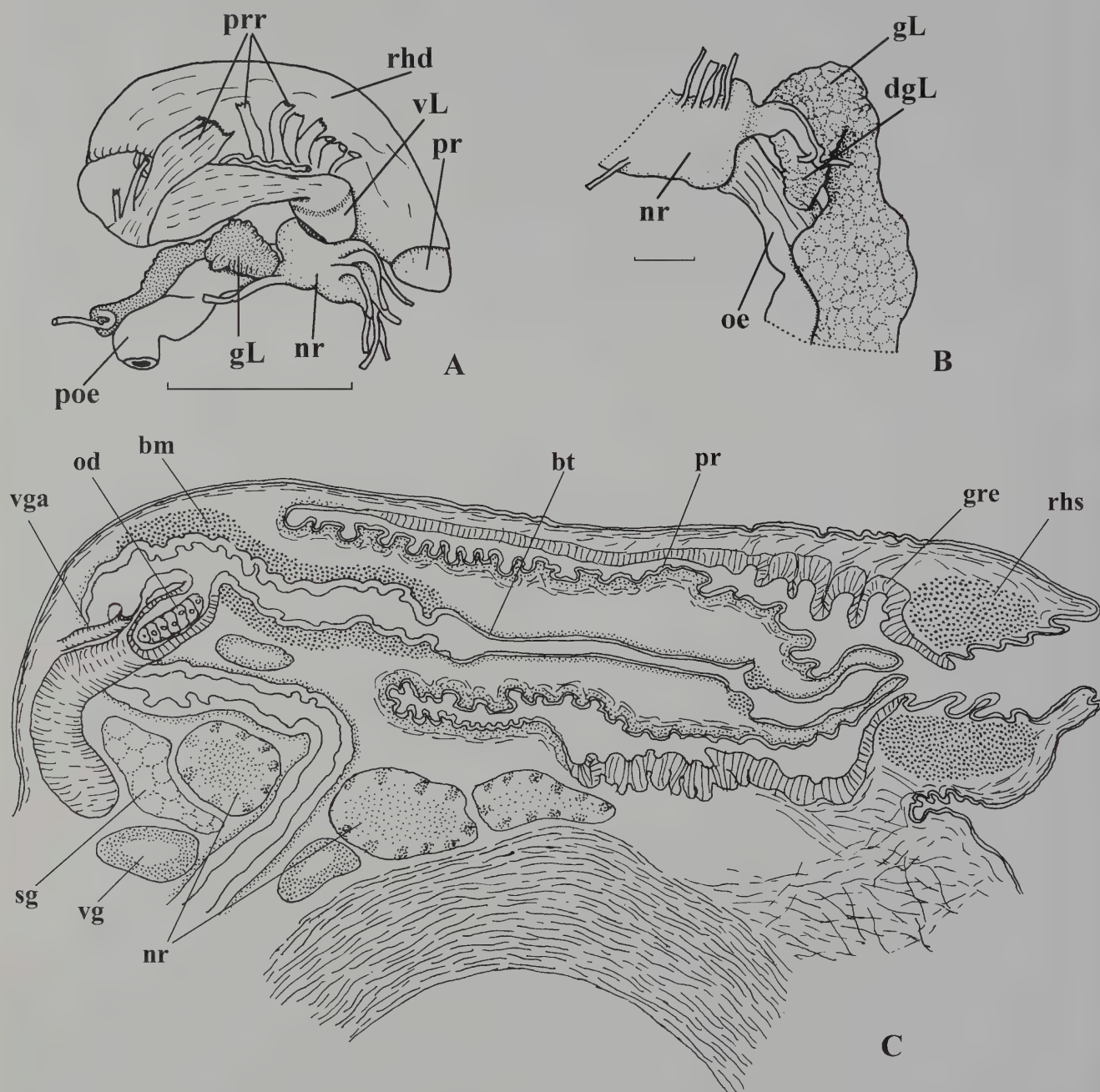
Figure 4. A,B — *Chlanidota (Pfefferia) palliata* (Strebel, 1908) (Buccinulidae) [after HARASEWYCH & KANTOR, 1999, modified from fig. 19]. A — right lateral view of anterior digestive system, salivary gland removed to show the valve of Leiblein. Scale 5 mm. B — Juncture of gland of Leiblein and oesophagus. Scale 2 mm. C — Semidiagrammatic longitudinal section through the anterior foregut of *Nquma scalpta* Kilburn, 1988 (Conoidea, Turridae, Crassispirinae) [after KANTOR, MEDINSKAYA & TAYLOR, 1997, modified from fig. 16]. Abbreviations: bm — buccal mass; bt — buccal tube; dgl — duct of the gland of Leiblein; gL — gland of Leiblein; gre — glandular epithelium of rhynchodaeum; nr — nerve ring; od — odontophore; oe — oesophagus; poe — posterior oesophagus; pr — proboscis; prr — proboscis retractors; rhd — rhynchodaeum (=proboscis sheath); rhs — rhynchostomal sphincter; sg — salivary gland; vg — venom gland; vga — duct of the venom gland; vL — valve of Leiblein.

The gland of Leiblein is very reduced or absent in many families — some Buccinidae (Volutopsiinae), all Melongenidae, Harpidae, Colubrariidae. When present it opens by a constricted duct either laterally or dorso-laterally in mid-oesophagus (Figure 4, A-B, dgL). The venom gland in Conoidea and midgut gland in some Marginellidae opens ventrally in the posterior part of the buccal cavity (TAYLOR *et al.,* 1993; PONDER, 1970; PONDER & TAYLOR, 1992) (Figure 4C, vga).

In the Tonnoidea there is the oesophageal gland, which is situated in the posterior part of the oesophagus, but it is situated ventrally and is a mere extension of the oesophagus, lined with dorso-ventral folds (KANTOR & HARASEWYCH, 2000).

3) Valve of Leiblein.
The valve (or pharynx) of Leiblein is the pear-shaped structure, marking the border of the anterior and mid-oesophagus. In the typical case it contains the cone valve of long cilia, although in some cases (e.g. some Buccinidae) the ciliary cone is absent.

A valve of Leiblein is found in all major groups of Neogastropoda, although it is often very reduced or absent. PONDER (1974) considered the position of the valve just posterior to the buccal cavity as plesiomorphic. It is situated in this position in the Cancellariidae (GRAHAM, 1966). The valve had never previously been found in Conoidea and this led KANTOR (1996) to suppose that it might originate twice in Neogastropod evolution.

Recently KANTOR & TAYLOR (this volume) found a very similar structure in two minute species of Conidae (subfamily Raphitominae) (*Kermia barnardi* and *Paramontana rufozonata*). It is situated immediately behind the buccal cavity, as in Cancellariidae. The venom gland bypasses the valve and opens anterior to the valve. The same situation is found in some Marginellidae, in which a long coiled gland, similar in general appearance to the conoidean venom gland is formed by the stripping off of glandular folds from the oesophagus (PONDER, 1970).

4) Anal gland.
The anal gland has been studied in detail for only a very few species, in particular in *Nucella lapillus* (FRETTER & GRAHAM, 1962; ANDREWS, 1992). In *N. lapillus* it "has the form of a group of caeca which unite with one another to form a duct leading to the rectum just within the anus" (FRETTER & GRAHAM, 1962: 233). The gland changes during the ontogeny and in young *Nucella* the gland is a simple diverticulum from the rectum, lined with simple ciliated columnar epithelium. Later the cells begin to develop small brown melanin granules.

In *Hormospira* (Pseudomelatomidae, Conoidea) the anal gland opens outside the rectum, also very close to the anus (KANTOR, 1988a). In this species the gland in the posterior part is lined with tall epithelium cells, containing the melanin granules (similar to that in *Nucella*). In the anterior part the epithelium is lower, although still having granules. In *Babylonia areolata* (Babyloniidae) there is a simple tubular gland, passing along the rectum and histologically similar to the anterior part of the anal gland in *Hormospira* (HARASEWYCH & KANTOR, this volume).

In some species of *Oliva* the anal gland has a dark coloration, indicating the presence of melanin granules, while in other it is very light and probably lacking the melanin (KANTOR & TURSCH, unpublished). An anal gland is absent in Buccinoidea.

Detailed examination of the structure of anal gland in a number of Neogastropodan families is still necessary.

Besides these generally accepted synapomorphies of Neogastropoda, two important apomorphies of the radula should be added:

5) Five or less teeth in a transverse row of the radula.
The number of the radular teeth in a transverse row is very variable in Neogastropoda. Five teeth are found in at least three unrelated families — Drilliidae (Conoidea), Olivellidae and Nassariidae. Four teeth are found in some Turridae (subfamilies Crassispirinae — KANTOR *et al.,* 1997; Cochlespirinae — KANTOR & SYSOEV, 1991). Three teeth in a row are characteristic for the majority of the Neogastropods, 2 teeth — for the family Conidae, and finally a single central tooth is present in Cancellariidae and majority of Volutidae.

6) Morphology and orientation of the lateral and marginal teeth in the radular sac, different from taenioglossan radula.
In taenioglossan radula the marginal and lateral teeth have a long "stalk" (according to the terminology of BANDEL, 1984) which is directed anteriorly, that is towards the radular bending plane, with the cusps on the tip, that are directed posteriorly, to the blind end of the radular sac (Figure 5A). In Neogastropoda the marginal and lateral teeth do not have a stalk and are directed posteriorly (Figure 5B-C).

There are two more characters, that are common for all the Neogastropoda, although they can not be considered as autapomorphies:

a. Proboscis with separate retractor muscles, inserted into the proboscis wall at the distal part. This character opposes Neogastropoda to some probosciferous Tonnoidea (Tonnidae and Ranellidae), in which proboscis is retracted due to the contraction of its muscular walls. In Pisanianuridae and Laubierinidae WARÉN & BOUCHET (1990) recorded long proboscis retractor muscles that pass through the nerve ring.

b. Position of the primary salivary glands "in front" of the circumoesophageal nerve ring (the ducts lie outside the nerve ring). This character is often used to oppose the probosciferous Tonnoidea, in which the salivary ducts pass through the nerve ring, to Neogastropoda. In some other "mesogastropods", e.g. some Littorinidae (*Bembicium, Risselopsis*) (REID, 1988), the ducts also lie outside the nerve ring and this character is not an autapomorphy of the Neogastropoda.

Thus Neogastropoda are well supported as a clade by at least 6 autapomorphies and some common characteristics and its monophyly is generally accepted, except by SHERIDAN, VAN MOL & BOUILLON (1973); SHIMEK & KOHN (1981) and GOLIKOV & STAROBOGATOV (1988).

FUNCTIONAL MORPHOLOGY AND MAIN EVOLUTIONARY TRENDS IN NEOGASTROPODA

All the above mentioned synapomorphies of neogastropods except one are confined to the digestive system, and to be more specific, to
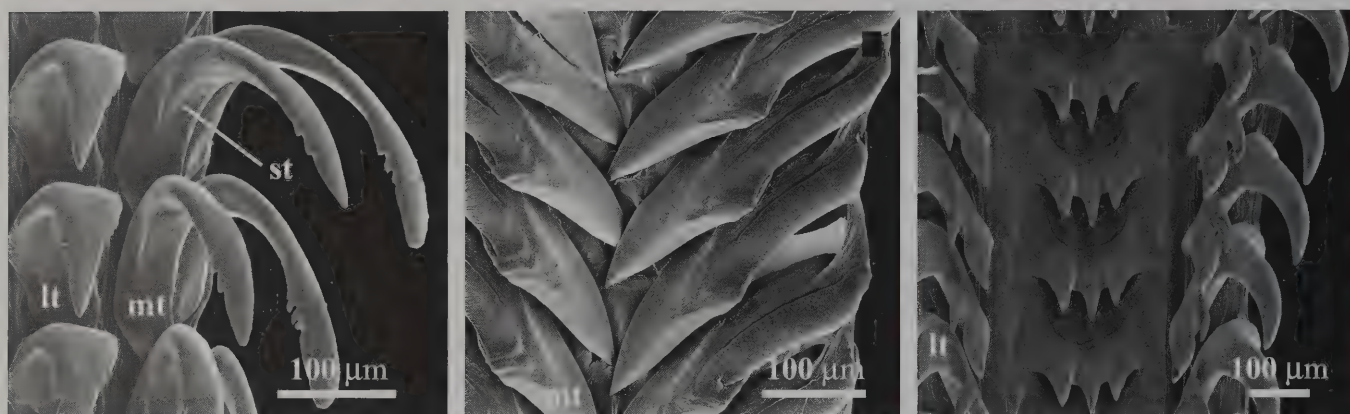
Figure 5. Lateral and marginal teeth of: A — Tonnoidea [*Obscuranella papyrodes* KANTOR & HARASEWYCH, 2000 — after KANTOR & HARASEWYCH, 2000, figure 22]. B — Conoidea [*Clionella sinuata* (Born, 1778)]. C — Buccinoidea [*Chlanidota (Chlanidota) densesculpta* (Martens, 1875) — after HARASEWYCH & KANTOR (1999), fig. 6A]. Abbreviations: lt — lateral tooth; mt — marginal tooth; st — "stalk" of the marginal tooth.

its anterior section. This is an important proof that origin and radiation of neogastropods was closely related to the main morphological transformations in the anterior foregut.

One of the most prominent characters that cause general morphological reorganisations of the foregut is the proboscis. Several different types and subtypes of proboscis have been described, two major — intraembolic (Conoidea) and pleurembolic (rest of neogastropods). [For the current review of proboscis morphology see BALL, ANDREWS & TAYLOR, 1997].

SHERIDAN, VAN MOL & BOUILLON (1973) as well as SHIMEK & KOHN (1981) considered the Conoidea with its characteristic intraembolic proboscis to have separate origin from the rest of the Neogastropoda due to major differences of the proboscis. Recently the questions of the homology of intraembolic proboscis of Conoidea and the pleurembolic proboscis were discussed by SIMONE (1999), who suggested the homology of both types.

The major difference between pleurembolic and intraembolic proboscis is the position of the buccal mass. In the former it is terminal, situated near the proboscis tip, while in the latter it is basal, situated at the proboscis base and in number of Conoidea even posterior to it. TAYLOR & MORRIS (1988), supposed, based on the anatomy of the proboscis of *Turricula nelliae* (Turridae) (in which the buccal mass is situated at the mid-length of proboscis), that the conoidean proboscis might originate from the pleurembolic by the shift of the buccal mass backwards. At the same time they did not gave any explanation for the possible reasons of backwards shifting of the buccal mass (with radula and normally developed odontophore, armed with the regular set of the muscles), which would prevent the radular protraction through the mouth and the contact with the prey. Failure to explain basal position of the buccal mass in Conoidea led KANTOR & SYSOEV (1990) to suggest an independent origin of intraembolic proboscis from the acrembolic one.

Later, additional neogastropods with the "intraembolic" proboscis were found, e.g. a representative of the family Pseudolividae, *Benthobia tryoni* (Figure 2C) (KANTOR, 1991). Finally, it appears that the basal position of the buccal mass is not unique for these mentioned groups, but is found in unrelated families — in Olivellidae (*Olivella*) (KANTOR, 1991), in all genera of the subfamily Ptychatractinae (usually attributed to Turbinellidae, but probably being the separate family) (Figure 7A) (KANTOR & BOUCHET, 1997; BOUCHET & KANTOR, 2000), in Mitridae (still unnamed species of *Eumitra*) (unpublished). In many neogastropods, the buccal mass occupies a mid-proboscis position, e.g. in Costellariidae (PONDER, 1972) and Marginellidae (GRAHAM, 1966). In Cancellariidae the buccal mass is situated at a significant distance from the mouth and only the tips of the very elongated radular teeth can be protruded through the mouth (HARASEWYCH & PETIT, 1984; 1986) (Figure 8A,C).

In my opinion the shift of the radular apparatus backward (as supposed for Conoidea by TAYLOR & MORRIS, 1988) does not have any reasonable functional explanation. In this case the radula, the main organ of prey capture, is moved from the mouth opening thus preventing its main functions. There is an opposite possibility that the basal position of the radular apparatus is a primitive state, inherited from the ancestor, while the terminal position of the buccal mass at the proboscis tip appeared in result of the secondary shift of the buccal mass.

Important evidence, that the basal position of the buccal mass is plesiomorphic for the neogastropods, is found in the arrangement of the radular musculature. In at least some Caenogastropoda lacking a proboscis and with the radula and buccal mass situated near the mouth, the radular sac is protruded backwards through the nerve ring (e.g. in Littorinidae — FRETTER & GRAHAM, 1962, fig. 17). Correspondingly, some radular muscles are situated posterior to the nerve ring and are attached to the floor of the body haemocoel. In Neogastropoda, with the formation of the proboscis, the radular sac was pulled anterior to lie **in front** of the ring. One may expect, that at least on initial stages of this transformation the muscles should pass through the ring to join the floor of body haemocoel. This condition is found in some Neogastropods, particularly in Drilliidae (Conoidea) (Figure 6, odrm) (SYSOEV & KANTOR, 1989), Pseudolividae and Olivellidae (KANTOR, 1991), as well as in Ptychatractinae (KANTOR & BOUCHET, 1997; BOUCHET & KANTOR, 2000) (Figure 7B, vodr). All these groups are characterised by short proboscis and basal buccal mass.

An intermediate stage is found in *Amalda* (Olividae) (KANTOR, 1991) and *Strictispira* (Strictispiridae, Conoidea)

(KANTOR & TAYLOR, 1994), where the radular muscles join the columellar muscle, but do not pass through the ring. In *Olivella*, some muscle branches are passing through the ring and some are bypassing it (KANTOR, 1996).

Finally in families which have a more or less long proboscis (apomorphic condition) and terminal buccal mass (e.g. in Buccinidae), the radular muscles in adults never pass through the nerve ring and are attached to the walls of the proboscis (WILLSMAN, 1943, fig. 2). Therefore we can observe the clearly directed sequence of morphological transformations — from short proboscis with basal buccal mass and radular muscles passing through the nerve ring to long proboscis with terminal buccal mass and muscles attached to the proboscis walls.

In the ontogeny of *Nucella* the embryo passes through stages, which are characterised by very short proboscis with a basal buccal mass and some radular muscles passing through the nerve ring (BALL *et al.,* 1997). Later these muscles are resorbed. Thus, despite striking differences in adult morphology between a proboscis with the basal buccal mass and a proboscis with a terminal buccal mass, the latter can be easily derived from the former.

Even so the initial basal position of the buccal mass, when radula could not used for prey capture should be explained functionally. KANTOR (1996) proposed the following evolutionary scenario. The establishment of the Neogastropoda as a clade was most probably connected with the development of the predatory mode of feeding. Active predation requires a shift of the mouth opening from the ventral position on the head (like in grazing gastropods) to a terminal position. This can be achieved by the formation of a proboscis or a snout as its first stage.

In all known predatory gastropods there is proboscis (introvert) of one of two basic types. Some of the predatory "mesogastropods" (Naticidae and Triphoridae) developed an acrembolic proboscis, which in the everted position brings the radula to the tip. In tonnoidean "mesogastropods" and neogastropods the elongation of the snout, which became retractable in its posterior part, formed the proboscis. The walls of the posterior part of the snout formed the rhynchodaeum, or proboscis sheath.

The fixation of the buccal mass in neogastropods at the proboscis
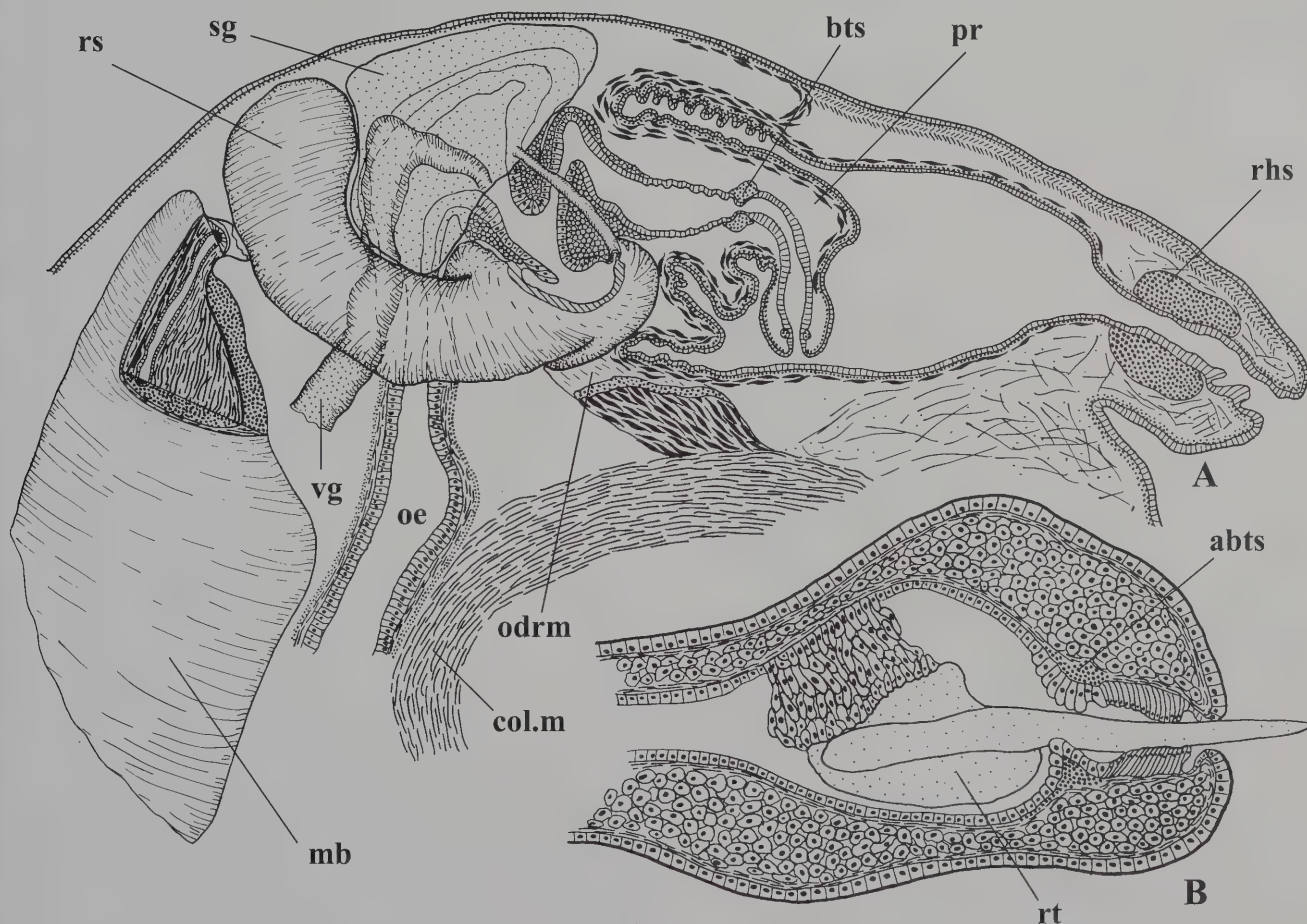
Figure 6. A — Semidiagrammatic longitudinal section through the anterior foregut of *Splendrillia chatamensis* Sysoev et Kantor, 1989 (Conoidea, Drilliidae). Nerve ring and large part of the venom gland are not shown; B — enlarged proboscis tip. [A, B — after SYSOEV & KANTOR, 1989]. Abbreviations: abts — anterior sphincter of the buccal tube; bts — intermediate sphincter of the buccal tube; col.m — columellar muscle; mb — muscular bulb of the venom gland; odrm — odontophoral retractor muscle; oe — oesophagus; poe — posterior oesophagus; pr — proboscis; prr — proboscis retractors; rhd — rhynchodaeum (=proboscis sheath); rhs — rhynchostomal sphincter; rs — radular sac; rt — radular tooth; sg — salivary gland; vg — venom gland.

base can be explained by a less complex transformation of the radular muscles, passing through the nerve ring, as well as by the possibility of capturing prey without using radula, if it is slow-moving (like some benthic worms) and swallowed whole, without tearing or rasping. Some Recent neogastropods, that can capture prey without using the radula are found among predatory Conoidea (genera *Caenodagreutes* — SMITH, 1967a, *Teretiopsis* — KANTOR & SYSOEV, 1989; many Terebridae — TAYLOR, 1990; and others), in which the radula and venom apparatus have completely disappeared (KANTOR & TAYLOR, this volume, figs. 15-16).

One of the ways of increasing the effectiveness of prey capture was further elongation of the proboscis. This allowed feeding on organisms, living in crevices and other shelters. The basal position of the buccal mass and initial arrangement of the radular and odontophoral muscles precluded further elongation of the proboscis, while disappearance of those branches of the muscles that passed through the ring allowed the anterior shift of the radular apparatus. Finally, the radula muscles become attached to the proboscis walls, thus eliminating any limitations for the elongation of the proboscis. Indeed, the neogastropod families with longest proboscis (Buccinidae, Vasidae, etc.) all have a terminal buccal mass. Among Recent neogastropods we can find different intermediate stages of the process of anterior shift of buccal mass and re-arrangement of radular musculature.

Prey capture, especially while the basal radular apparatus has reduced mobility, can be significantly facilitated with some kind of the mechanism of prey immobilisation. The use of a toxic secretion became such a mechanism and this explains the origin of the second pair of salivary glands (accessory salivary glands). In the ontogeny of *Nucella* the glands form as paired invaginations on ventral proboscis lip (BALL *et al.*, 1997). Thus, they are not connected with the anterior gut and open into anterior buccal tube despite any transformation of the foregut.

There is only one investigation of the chemical composition of the secretions from accessory salivary glands in Neogastropoda (ANDREWS, ELPHICK & THORNDYKE, 1991). It was demonstrated
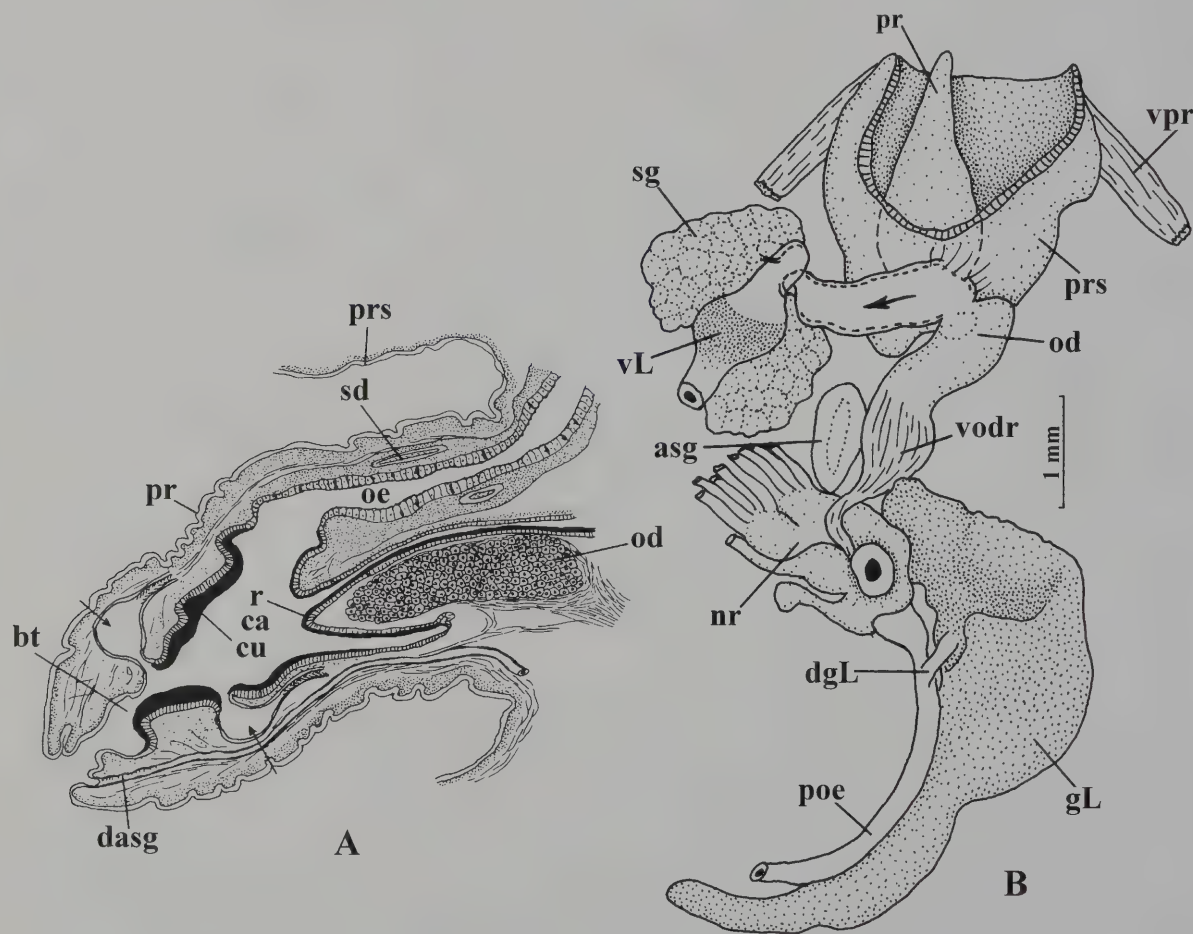
Figure 7. Anatomy of the anterior digestive system of Ptychatractidae. A — longitudinal semidiagrammatic section of the proboscis of *Latiromitra barthelowi* (Bartsch, 1942) [after BOUCHET & KANTOR, 2000, modified from fig. 2]. Arrows mark the enlargement of the salivary ducts ("ampullas"). B — anterior digestive system of *Exilia.* Anterior oesophagus cut in front of the nerve ring to show the passage of ventral odontophoral retractor through the ring. Abbreviations: asg — accessory salivary gland; bt — buccal tube; ca — buccal cavity; cu — cuticular lining of the buccal cavity; dasg — duct of accessory salivary gland; dgL — duct of gland of Leiblein; gL — gland of Leiblein; nr — nerve ring; od — odontophore; oe — oesophagus; poe — posterior oesophagus; pr — proboscis; prs — rhynchodaeum (=proboscis sheath); r — radula; sd — salivary duct; sg — salivary gland; vL — valve of Leiblein; vodr — ventral odontophoral retractor; vpr — ventral proboscis retrac-

that the secretion in *Nucella lapillus* causes flaccid paralysis in prey. As it was said above, the ducts of the gland are lined with non-ciliated epithelium, while the wall of the gland contains the circular layer of muscle fibres. It seems likely, that contraction of the muscle fibres causes the propulsion of the secretion through the duct.

Although even with a basal buccal mass feeding is possible (as is demonstrated by presence of different Recent neogastropods with basal buccal mass), we have no data on the feeding mechanisms of many of them. Nevertheless, we can observe different ways of morphological and functional transformations among recent neogastropods, that allow the use of the radula for feeding, that "overcome" the plesiomorphic basal position of the buccal mass.

In Cancellariidae, the lateral and marginal teeth are lost, while the central teeth are greatly elongated so that their tips can protrude through the mouth opening (Figure 8). For some Recent representatives of Cancellariidae, parasitism on fishes and echinoderms has been demonstrated (O'Sullivan, McConnaughey & Huber, 1987; Buck, 1991). Thus, the radula is probably used for penetration of the prey skin, and only the tips of the teeth are in contact with the prey (Petit & Harasewych, 1986).

Most striking and well studied are the feeding mechanisms of Conoidea. In Conoidea the venom apparatus was developed, which consists of the venom gland and muscular bulb. The evolution of the venom apparatus greatly improved prey capture through the envenomation, and allowed the retention of the primitive proboscis type with the basal buccal mass in most groups of Conoidea. At a very early stage of evolution of the group, the specialised mechanism of using the detached marginal teeth at the proboscis tip for stabbing the prey appeared (Sysoev & Kantor, 1987; Kantor & Taylor, 1991). Nevertheless, even within Conoidea, different ways of overcoming the basal position of the buccal mass can be found. In some non-related genera, the buccal mass is shifted forward and the radula can be protruded through the mouth opening (e.g. *Turricula, Toxiclionella, Strictispipra* — Kantor & Taylor, 1994). In several conoidean taxa the buccal mass itself is able to evert through the mouth, which allows the direct use of the radula for prey capture (Taylor *et al.*, 1993) (Figure 9 — *Funa jeffreysii*). In some Clavatulinae (Turridae) with an anteriorly shifted buccal mass the posterior part of the rhynchodaeum can evert and this makes the proboscis similar in many respects to that of pleurembolic type (including a secondary elongation of the oesophagus between buccal mass and nerve ring).

Finally, in the majority of Neogastropoda the buccal mass was shifted to the proboscis tip and the radula became the primary organ for prey capture. In these molluscs, the radular muscles mostly lost the connection to the columellar muscle and became attached to the walls of the proboscis.

In summary we can suppose that the most primitive neogastropods are characterised by a short proboscis with a basal buccal mass and at least some of the radular muscles passing
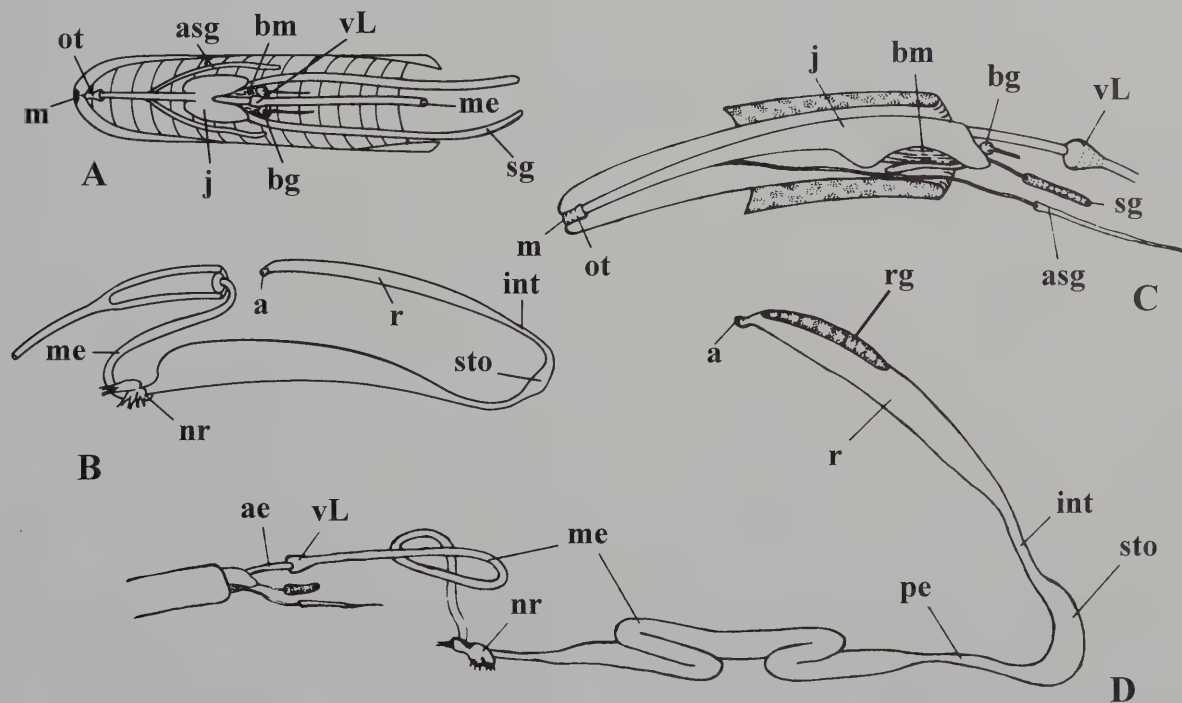
Figure 8. Digestive system of Cancellariidae: A, B — *Admete viridula* (Fabricius, 1780) [after Harasewych & Petit, 1986, modified]. C, D — *Olsonella smithi* (Dall, 1888) [after Harasewych & Petit, 1984, modified]. A — dissection of anterior portion of proboscis, opened mid-dorsally. B — diagrammatic representation of the digestive system. C — dissection of retracted proboscis, viewed from left side. D — diagrammatic representation of the digestive system. Abbreviations: a — anus; ae — anterior oesophagus; asg — accessory salivary gland; bg — buccal ganglia; bm — buccal mass; int — intestine; j — jaw; m — mouth; me — mid-oesophagus; nr — nerve ring; or — oral tube; pe — posterior oesophagus; rg — anal (rectal) gland; sto — stomach; vL — valve of Leiblein.
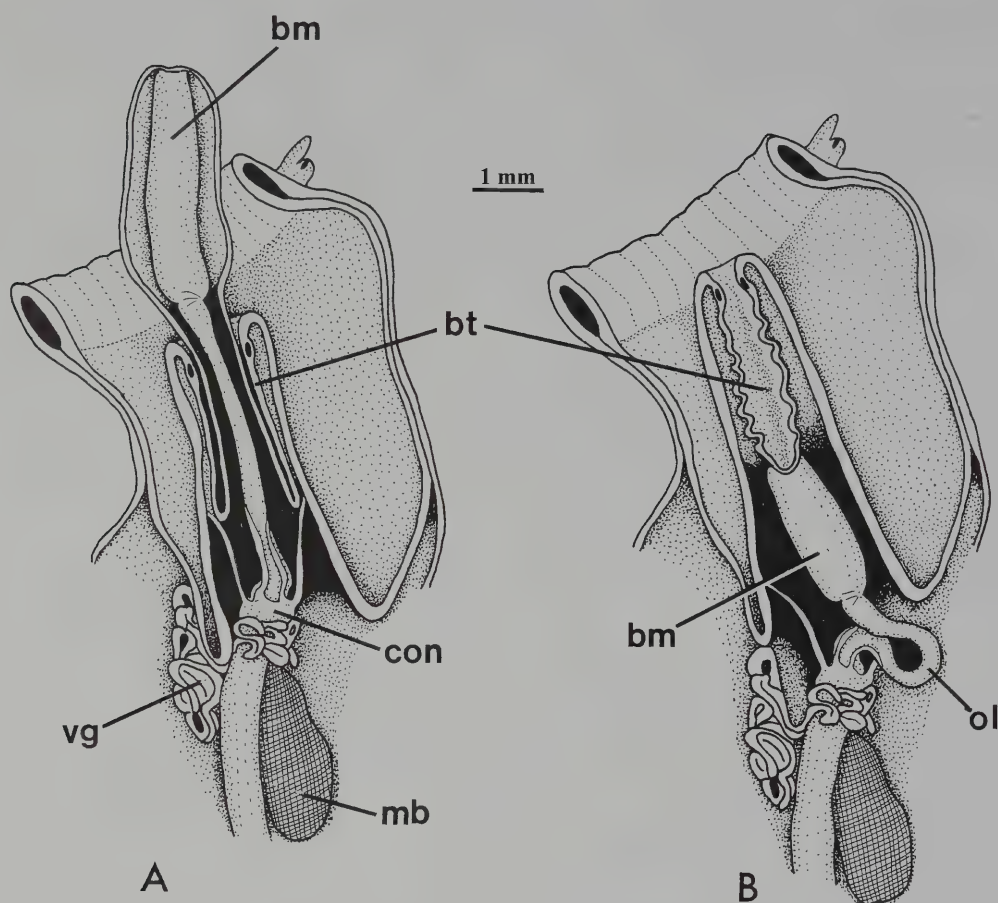
Figure 9. Dissected anterior digestive system of *Funa jeffreysii* (Smith, 1885) (Conoidea, Turridae). A — buccal mass in everted position; B — buccal mass in retracted position. After TAYLOR, KANTOR & SYSOEV, 1993. Abbreviations: bm — buccal mass; bt — buccal tube; con — nerve ring; mb — muscular bulb of the venom gland; ol — oesophageal loop; vg — venom gland.

through the nerve ring. Among Recent neogastropods there are at least 3 taxa that fulfil these requirements: some Conoidea (family Drilliidae), family Pseudolividae and family Ptychatractidae. Although the former have the less derived radular type with 5 teeth in a transverse row, the two latter ones possess a less derived anterior foregut, that is possession of the simple gland of Leiblein and a well-developed valve of Leiblein, the main autapomorphies of the neogastropods.

OVERVIEW OF HYPOTHESES ON THE ORIGIN OF THE NEOGASTROPODA

Among different hypotheses on the origin of the Neogastropoda four are in common use (in chronological order).

1. Derivation from the probosciferous higher mesogastropods in the families of the Tonnoidea.

This hypothesis was proposed by AMAUDRUT (1898) and supported by GRAHAM (1941). Currently BANDEL and RIEDEL support this point of view. RIEDEL (1994, 2000) actually included Ficidae in neogastropods, considering the Tonnoidea their sister group.

A detailed description of the anatomy of Ficidae is still lacking. In many respects it is very different from the rest of Tonnoidea and it probably does not belong here. The author had the opportunity to dissect a single frozen specimen of *Ficus*. It has very little resemblance to any neogastropod and seems to have an acrembolic type of the proboscis, while all other neogastropods have the pleurembolic one. More information is necessary to clarify the taxonomic position of *Ficus*.

The adult foregut morphology is very different in Tonnoidea and Neogastropoda. Detailed studies of different Tonnoidea are available: that of *Tonna* (WEBER, 1927), of *Cymatium* and *Bursa* (HOUBRICK & FRETTER, 1969), of an Antarctic ranellid *Obscuranella* (KANTOR & HARASEWYCH, 2000).

The tonnoidean proboscis is characterised by very small terminal buccal mass and radular muscles attached to the proboscis wall. Therefore the plesiomorphic proboscis of the neogastropods with the basal buccal mass can not be derived from that of Tonnoidea. Moreover most Tonnoidea do not have proboscis retractors (see above). Instead, the contraction of the proboscis is achieved by the contraction of the proboscis walls.

There are some structures well developed in Tonnoidea, but absent in Neogastropoda, particularly the presence of the lateral longitudinal folds in oesophagus; the bilobed salivary glands, which have separate ducts for each lobe, that fuse together; the well developed jaws and oesophageal gland. So, for the moment we do not have any synapomorphies for Tonnoidea and neogastropods. In the paleontological record Neogastropoda appeared earlier than Tonnoidea (TRACEY, TODD & ERWIN, 1993).

One can suppose that Recent Tonnoidea and neogastropods may have some remote common predatory ancestor, possessing a very short proboscis, but so far we do not have any morphological proof and such an assumption is a mere speculation.

2. Derivation from an archaeogastropod or primitive lower mesogastropod (Ponder, 1974).

This hypotheses was analysed and rejected by TAYLOR and MORRIS (1988) and by PONDER and LINDBERG (1997). The supposition of Ponder was based mainly on the facts, that neogastropods share similar structures with archaegastropods, but not with higher mesogastropods; namely the accessory salivary glands and anal gland. Accessory salivary glands were thought to be present in Acmaeidae, but later it was proven that these structures are not homologous. Similarly, the rectal pouches of some archaegastropods appear not to be homologous with the anal gland of neogastropods (ANDREWS, 1992). Moreover, HASZPRUNAR (1985) showed that synapomorphic characters of osphradial structure are shared by higher meso- and neogastropods.

3. Independent origin of Bucciniformii and Coniformii from different archaeogastropod ancestors.

This less-known hypothesis, proposed by GOLIKOV & STAROBOGATOV (1988), was based on a completely original theory of radular transformations in evolution and ontogeny of gastropods. In the ontogeny of the radulae of chitons and pulmonate gastropods (KERTH, 1983) a subdivided radular plate is formed initially. Later in some groups this plate is divided into two large teeth, which GOLIKOV & STAROBOGATOV named "initial" teeth. According to their view, the position of these initial teeth differs markedly among groups. Thus, in taenioglossan radulas they are the first pair of lateral teeth, while in rhipidoglossan radulas they can be the first laterals, or the 6th pair of laterals. After formation and splitting of the initial teeth the "rachidian" tooth is formed between them. It is marked in radulae formulas as "R".

In other groups, the initial teeth fuse to form a central tooth (marked as "C"). GOLIKOV & STAROBOGATOV provided a complex scheme of radulae transformation in different gastropod groups. For the purpose of current presentation it is important, that they distinguish two types of median tooth — central and rachidian. GOLIKOV & STAROBOGATOV provided several neogastropod radulae formulae:

Cyclope (Nassariidae): M — : — (L) — C — (L) — : — M,

where M — marginal; L — lateral (position in brackets indicate the reduction of the tooth); C — central; : — position of the longitudinal bent of radular membrane.
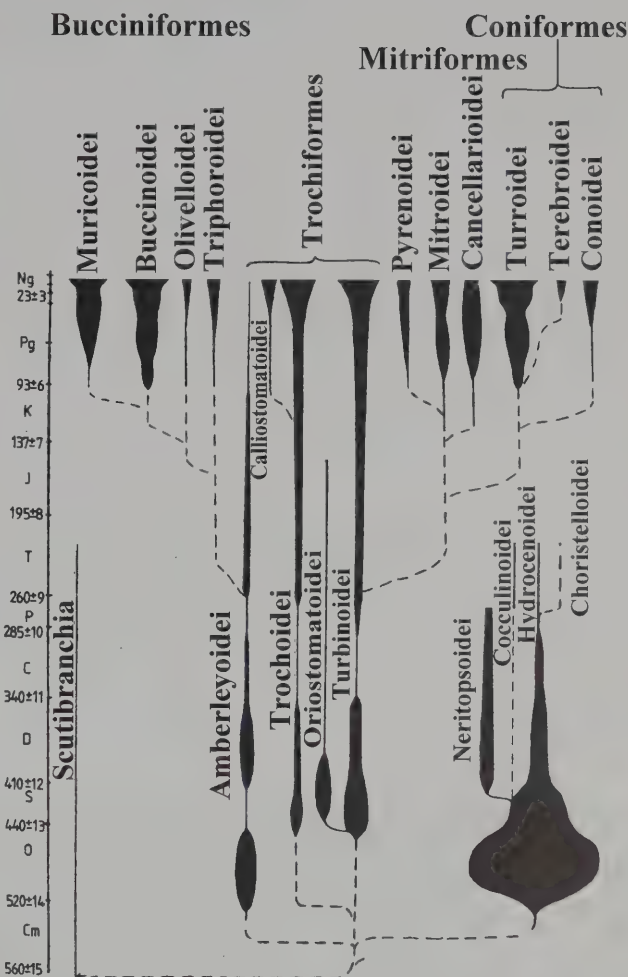
Figure 10. Hypothesis on the phylogenetic relationships of Neogastropoda, after GOLIKOV & STAROBOGATOV, 1988 (modified).

Olivella (Olivellidae): (M) — M — : — C — : — M — (M)
Buccinum (Buccinidae): M — : — C — : — M
Marginella (Marginellidae): C
Pseudomelatoma (Pseudomelatomidae): M — : — R — : — M
Fasciolaria (Fasciolariidae): I — : — R — : — I
Anachis (Columbellidae): I — : — (R) — : — I

Thus, median teeth in Bucciniformes and Coniformes appeared according to GOLIKOV & STAROBOGATOV not to be homologous. So, they supposed that Bucciniformii (that are majority of the neogastropods, including Triphoridae) and Coniformii (in which they included besides Conoidea also Mitridae, Cancellariidae and Pyrenoidea) originated independently, the former ones from Amberleyoidei while the latter from Turbinoidei (Figure 10).

This hypothesis ignores the large number of synapomorphies of both groups recognised by GOLIKOV & STAROBOGATOV and therefore supposes the independent origin of accessory salivary

glands, rectal gland, dorsal mid-gut gland, etc. Besides, studies of radular ontogeny in *Buccinum undatum* and *Volutomitra groenlandica alaskana* did not reveal any signs of the "initial" teeth. On the contrary, the median tooth was formed as an unpaired structure, which soon attains its final shape (KANTOR, 1988b).

4. **The cladistic analysis of morphology of gastropods by PONDER & LINDBERG (1997).**

In the summary cladogram of the gastropods, obtained by PONDER & LINDBERG (1997) on the basis of morphological characters, Buccinidae came out as the sister taxon to the rest of the neogastropods. The sister group of Neogastropoda itself is a certain member of the Sorbeoconcha above the level of cerithioideans (Figure 1C).

According to discussion above concerning the foregut anatomy of the underived neogastropod, it is obvious that Buccinidae cannot be the most primitive family of neogastropods, since they possess the most advanced proboscis structure. Such a position of Buccinidae in the cladogram may be a result of the process of coding the characters. It is stated, that during the analysis the accessory salivary glands and rectal gland were not considered as secondarily lost in the process of evolution, but as initially absent.

To support their point of view, PONDER and LINDBERG stated, that Buccinidae have the caecum of the stomach, which is a plesiomorphic character, absent in many neogastropods. This statement is incorrect. First, in many of the neogastropods the stomach caecum (usually referred to as *posterior mixing area*) is present. It is well developed and large, e.g. in *Oliva, Olivella,* Muricidae and many others. It is also absent in some Buccinidae (e.g. *Colus gracilis* — SMITH, 1967b) and closely related Buccinulidae (HARASEWYCH & KANTOR, 1999) and Fasciolariidae (unpublished). Secondly, there is no proof, that the posterior mixing area in neogastropods is homologous with the caecum of mesogastropods.

A serious problem in understanding neogastropod origin and radiation is the incongruence between morphological data and palaeontological records. From the morphological standpoint, Buccinoidea should be the most derived neogastropods, possessing long proboscis with terminal buccal mass and lacking the accessory salivary and rectal glands (obviously secondary loss). At the same time, Buccinidae and Fasciolariidae are the first families of the neogastropods, found in fossil record (TAYLOR, MORRIS & TAYLOR, 1980; TRACEY, TODD & ERWIN, 1993).

Nevertheless, there is high uncertainty in attributing early Cretaceous fossil neogastropods to current families. Unfortunately the mentioned oldest records are based on unpublished materials of
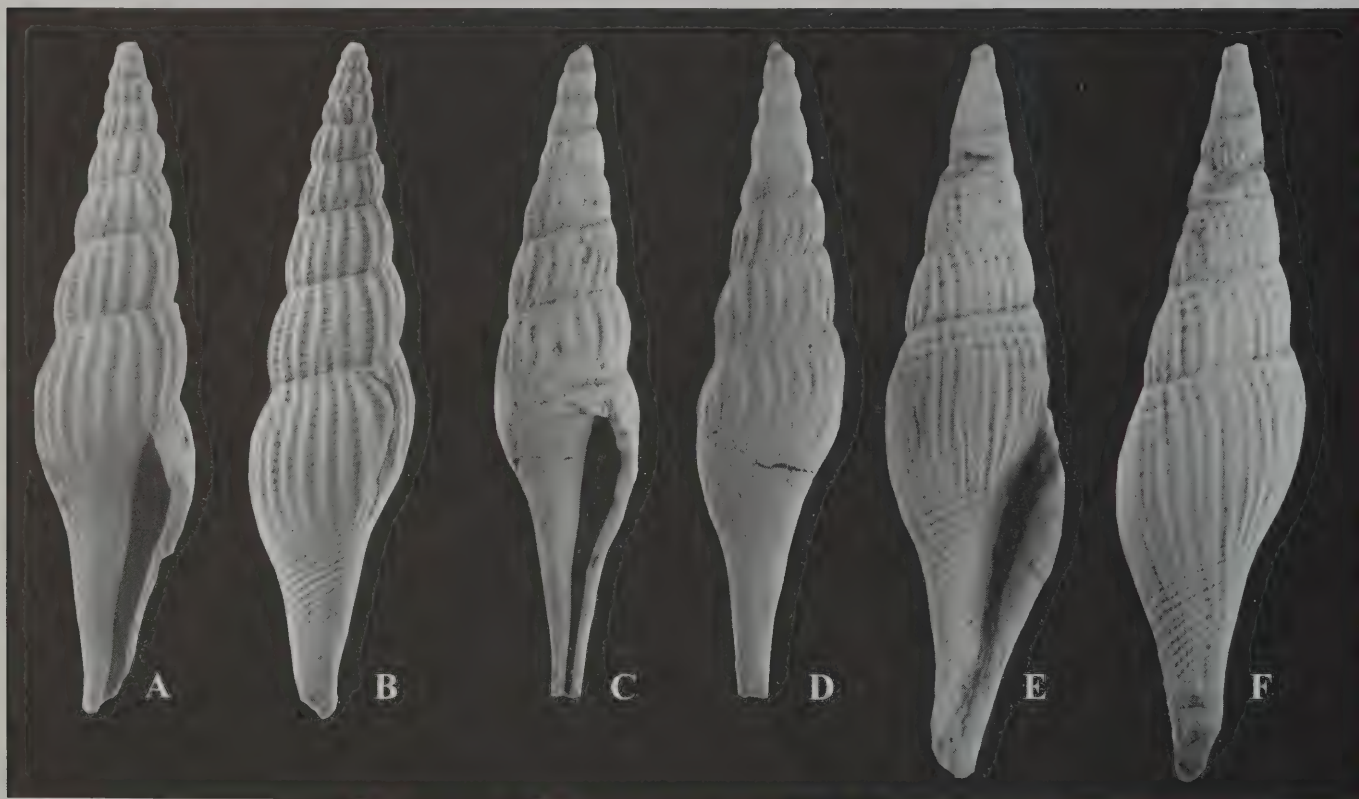
Figure 11. Fossil members of the genus *Exilia* (Ptychatractidae), erroneously attributed to Fasciolariidae. **A-B** — *Exilia pergracilis* Conrad, 1860. Lower Eocene, Midway Group, near Oak Hill, Alabama, specimen illustrated by Bentson, 1940: pl. 1, figs 9-10 (Museum of Paleontology, University of California, Berkeley,UCBMP 11623), 14.8 mm. **C-D** — *Exilia lincolnensis* Weaver, 1916. Upper Eocene, Lincoln Creek Formation, Porter Bluff, Washington, (The Burke Museum, University of Washington, UWBM 19936), 30.5 mm. *Exilia terebriformis* Stephenson, 1941, type-species of *Graphidula,* Maastrichtian, Nacatoch Sand, near Chatfield, Navarro County, Texas, holotype (National Museum of Natural History, Smithsonian Institution, Washington, DC, USNM 77085), 46.0 mm. All photos — courtesy of Dr. Anton Oleinik.

Noel Morris and therefore can not be checked. In the discussion of the oldest fasciolarid species ("*Fusus*" *valangiensis* Pictet & Campiche, 1872) TRACEY *et al.* (1993) cited the personal communication of N. Morris: "Such early fusiform gastropods may belong to a stem group of the paraphyletic Fasciolariidae and the Turridae". As it is known from morphological data these are totally unrelated groups.

The other example of erroneous allocations to the family Fasciolariidae is the fossil genus *Exilia* Conrad, 1860 (Figure 11). CONRAD (1860) originally did not assign *Exilia* to any family, but later placed it in the Pleurotomidae [= Turridae] (CONRAD, 1865). Several authors followed this view, e.g. WENZ (1943), and with doubts also POWELL (1966). Alternatively, the genus has been classified in various buccinoid families: Fasciolariidae (HICKMAN, 1980), Fusinidae (BENTSON, 1940), Buccinidae (THIELE, 1929). Finally MAXWELL (1988) correctly placed *Exilia*, together with *Graphidula*, in the subfamily Ptychatractinae of the Turbinellidae, which as was mentioned already, have most primitive foregut anatomy.

Therefore, many of the fossil Cretaceous Neogastropoda still await for critical reassessment of their taxonomic position and there is still no certainty, which representatives of which families are the oldest in paleontological records.

CONCLUSIONS

The most primitive neogastropods were characterised by a short proboscis with a basal buccal mass and radular retractors passing through the nerve ring and joining the columellar muscle, possessing a valve of Leiblein, the anal and accessory salivary glands. There are several groups, that fulfil these requirements, particularly Conoidea (families Drilliidae and Turridae), Pseudolividae and Ptychatractidae. The Pseudolividae and the anatomically close Ptychatractidae have less modified mid-gut glands than Conoidea.

We should seek for a sister group of Neogastropoda among carnivorous groups of Caenogastropoda, or more specific Sorbeoconcha, with an underived foregut. More studies of the anatomy of primitive Tonnoidea are necessary before we can either support or disprove the possibility of them being the sister group of the Neogastropoda.

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Evaluation of character state polarity of *Conus* radular tooth characters

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KEY WORDS: Conoidea, Conidae, *Conus*, radular tooth, character state, polarity, plesiomorphism, apomorphism.

ABSTRACT The character state polarity of fifteen characters of *Conus* radular teeth is evaluated by analysing a large data set (1400 radular teeth from 450 *Conus* populations of specific and subspecific rank) from previously published as well as unpublished results. A selected sample of radular teeth in different developmental stages is employed here to exemplify the characters and the results of the analysis. The selected sample is representative of putative primitive, generalist, vermivorous, molluscivorous and piscivorous type of teeth occurring in species of the genus *Conus* and includes radular teeth information for some species of older and more recent turrids that likely represent out-group and/or sister groups. Based on the state of the characters in putative ancestral species and, where available, on the evidence provided from the intra-specific ontogenetic change observed, plesiomorphy or apomorphy of each character are determined.

RIASSUNTO Viene analizzato lo stato di quindici caratteri del dente radulare del genere *Conus* L. Lo studio di oltre 1400 radule da 450 popolazioni (*species* e *sub*-species) di *Conus* costituisce l'ampia base di dati indispensabile per tale analisi. Per esemplificare i caratteri e riepilogare i risultati di questa analisi, viene utilizzata una selezione di denti radulari in differenti stadi di sviluppo. Il campione è rappresentativo dei diversi tipi morfologici osservati in *Conus* spesso correlabili alle specializzazione trofica specifica e qui classificati come "primitivo", "generalista", "vermivoro", "molluscivoro". Il campione include il dente radulare di alcune specie di turridi, potenziale *out-group* e/o *sister groups* in una analisi cladistica. Lo stato plesiomorfico o apomorfico di ciascun carattere viene infine determinato confrontando lo stato del carattere in specie ritenute ancestrali in base ad altri caratteri morfologici, al *record* fossile dove noto e sull'evidenza fornita dalle modificazioni ontogenetiche osservate a livello intraspecifico.

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INTRODUCTION

Turridae and Conidae, here considered as two distinct groups according to the classic systematic arrangement, are among the richest families in species number, have a relatively high density in the majority of their populations and most species live in shallow water. These attributes offer good opportunities for a systematic study of the radula.

The study of the radular tooth in the venomous genus *Conus* L. started already in the second third of the XIX century, but only in the last third of the XX century it increased notably. In the recent years many studies on *Conus* radular tooth were published.

This was probably due to several factors as, for instance, to an increased interest and knowledge of collectors for this group, to an increased facility to collect material from previously less accessible localities, to the interest of biologists for the intriguing biochemical properties of *Conus* venom. Though a primary aim of some authors has been the potential use of difference in the radular teeth for taxonomic purpose and in species separation, the evolutionary history underlying *Conus* biology may help clarifying the systematic of this large group and understanding functional aspects of the complex mechanisms by which species in this taxonomically difficult genus envenomate their prey, defend from predators and deter competitors.

In the course of previous studies, important differences between juvenile and adult individuals of some *Conus* species were pointed out, thus demonstrating an ontogenetic evolution of the radular tooth and arising a special attention.

A phylogenetic hypothesis is not yet available for *Conus* and molecular data have been assembled only for a limited number of species (Duda & Palumbi,1999; Espiritu *et al.*, 2001) mostly from a single geographic area (i.e. the Indopacific marine province) and generally for shallow-water species. Thus a phylogenetic scheme for the radular tooth based on qualitative and quantitative characters observed in large series of *Conus* teeth, would be desirable.

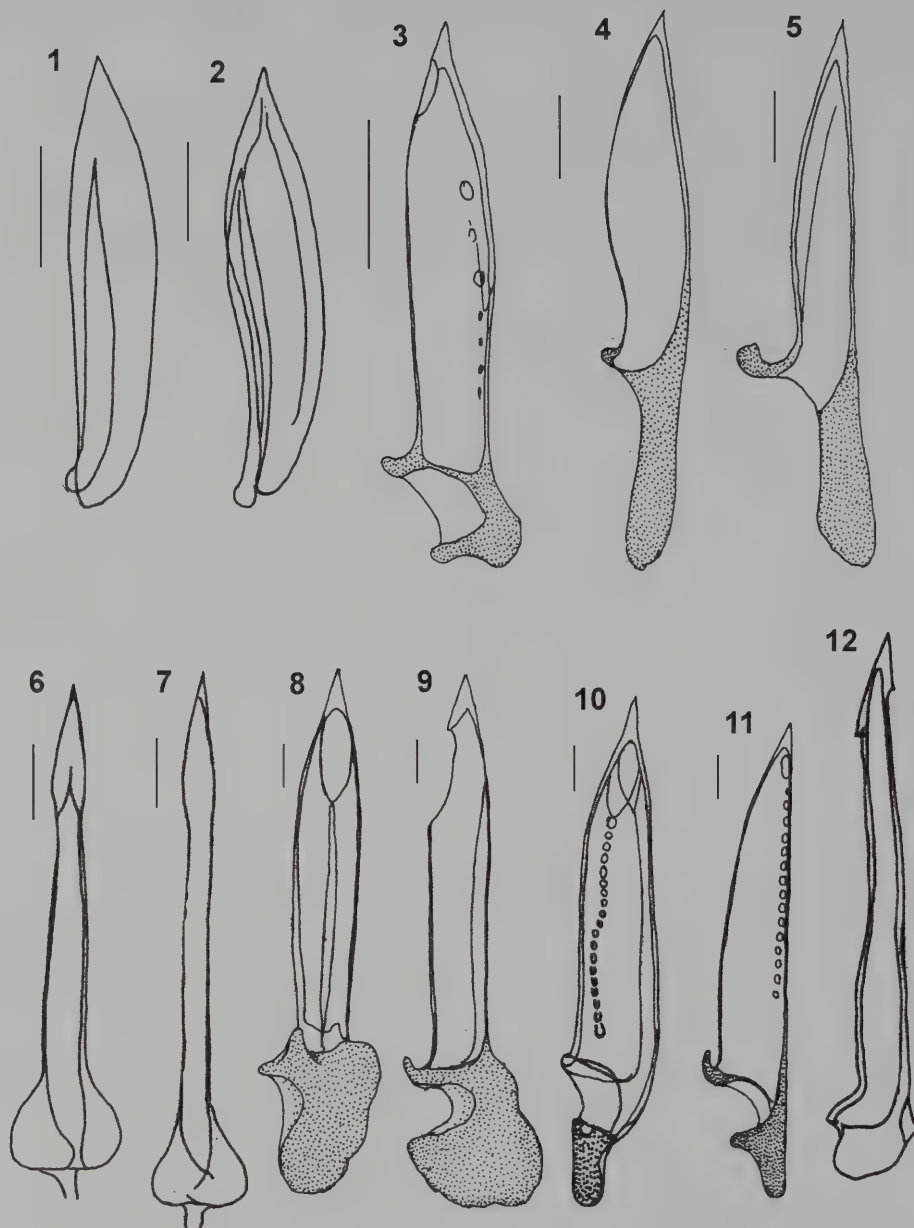
References to previous studies on the radular teeth of *Conus* can be found in Rolán (1992, 2000) and Kohn, Nishi & Pernet (1999).

Based on the examination of the largest sample of radular teeth of Conidae and Turridae recently attempted and on the observation of ontogenetic changes in several species, we could infer how some of these changes may have occurred during the about 55 million years evolution (Kohn,1990, Espiritu *et al.,* 2001) and we are now in the condition to define a possible state of the main characters and their polarity.

Most of the characters here studied have been discussed in Rolán (1992), Rolán & Raybaudi Massilia (1994a) and in Rolán & Boyer (2001) along with the study of the ontogeny of *Conus ermineus*.

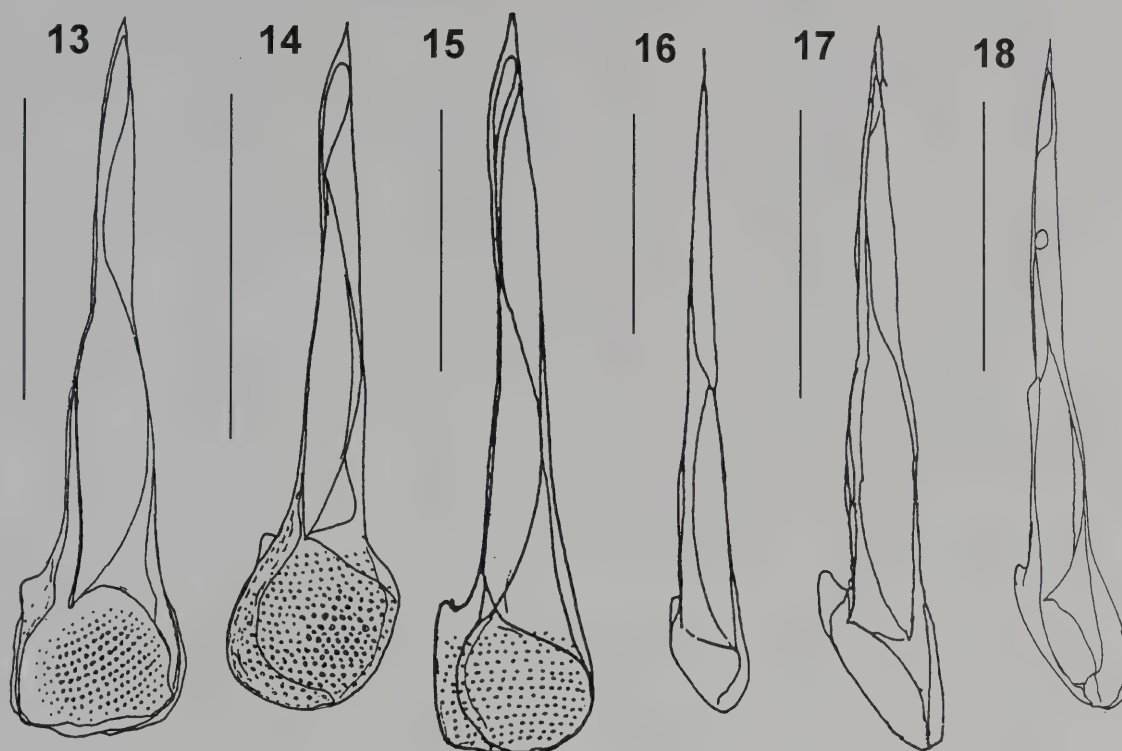
MATERIAL AND METHODS

The authors studied more than 1400 radulae of *Conus*, collected worldwide from a wide range of depth, including at least 350 species or taxa of specific or sub-specific rank. When different populations of a single species are considered and including information from literature, we have knowledge of the radular tooth morphology of about 450 populations of *Conus*. Additionally, in order to compare putative primitive state of the characters, the radular tooth of 55 species of Turridae were included in this study.

Radular teeth from several growth series of *Conus* species were studied and their ontogenetic change was observed. Though the "ontogenetic rule" is not acceptable as a general rule, it proved to

Figs. 1-12. Radular tooth of some Turridae species. Scale bar 0.01 mm. Fig. 1: *Crassispira callosa*, shell length 28.8 mm, Miamia, Ghana (from Fernandes, Rolán & Otero-Schmitt, 1995). Fig. 2. *Crassispira funebralis*, shell length 28.8 mm, Farol das Lagostas, Angola (from Fernandes, Rolán & Otero-Schmitt, 1995). Fig. 3. *Mangelia merlini*, shell length 7.0 mm, P. Cansado, Mauritania (from Rolán & Otero-Schmitt, 1999). Fig. 4. *Mangelia pontyi*, shell length 4.0 mm, Luanda, Angola (from Rolán & Otero-Schmitt, 1999). Fig. 5. *Mangelia albilonga*, shell length 8.0 mm, Luanda, Angola (from Rolán & Otero-Schmitt, 1999). Fig. 6. *Mitrolumna monodi*, shell length 4.2 mm, Dakar, Senegal (from Rolán & Boyer, 2001). Fig. 7. *Mitrolumna saotomensis*, shell length 4.0 mm, São Tomé (from Rolán & Boyer, 2001). Figs. 8-9. *Mangelia congoensis*, shell length 3.0 mm, Luanda, Angola (from Rolán & Otero-Schmitt, 1999). Figs. 10-11. *Mangelia digressa*, shell length 4.1 mm, Luanda, Angola (from Rolán & Otero-Schmitt, 1999). Fig. 12. *Benthofascis* sp. (from Powell, 1966).

be extremely useful to confirm some important trends and to support our determination of characters state polarity.

Thus, starting from the state of the character in putative outgroup and sister-group species of turrids as well as in putative oldest species of *Conus* (according to shell morphology and information on shells from the fossil data in THIELE, 1929-31, PETUCH, 1988, KOHN, 1990) and by analysing either inter-specific variation and intra-specific ontogenetic changes, we selected a set of characters believed to be of essential complementation to other morphological, anatomical and molecular characters for working out a phylogenetic analysis of the genus *Conus.*

Previously published works (ROLAN, 1992, 1993, ROLAN & RAYBAUDI MASSILIA, 1994a, 1994b, and ROLAN & BOYER, 2000) and personal unpublished observations include the wide body of

Figs. 13-18. Radular tooth of some Conus species. Scale bar 0.1 mm. Fig. 13. *Conus trovaoi*, shell length 32.5 mm, Limagens, Angola (from Rolán & Röckel, 2000). Fig. 14. *Conus neoguttatus*, shell length 29.1 mm, Santa Maria, Angola (from Rolán & Röckel, 2000). Fig. 15. *Conus naranjus*, shell length 22.7 mm, Santa Maria, Angola (from Rolán & Röckel, 2000). Fig. 16. *Conus elegans*, shell length 29.2 mm, Aden Gulf, N. Somalia. (from Rolán & Raybaudi Massilia, 1994). Fig. 17. *Conus stocki*, shell length 26.7 mm, Masirah, Oman (from Rolán & Raybaudi Massilia, 1994). Fig. 18. *Conus lizarum*, shell length 20.8 mm, N. Somalia (from Rolán & Raybaudi Massilia, 1994).

information, quantitative and qualitative determination of the several descriptors of *Conus* radular morphology. Though KOHN *et al.* (1999) recently reviewed a small part of our data set and proposed nomenclature adjustments of some terms used, for practical convenience and for the purpose of this paper we prefer to maintain the acronyms employed in our previous works.

The terms used to define these characters were introduced by TROSCHEL (1866), and later employed and increased by BERGH (1895), WARMKE (1960), NYBAKKEN (1970) and others (see KOHN *et al.*, 1999 for a hystorical review). ROLÁN (1992) used some ratios for radular teeth in species separation. KOHN *et al.* (1999) adopted most of these characters translating the terms into English and adding some new ratios. We prefer to maintain our original terms: DR was translated to TL, LC to SL. etc., a change which does not represent an important contribution. Furthermore, we did not employ some of these more recent parameters introduced by KOHN *et al.* (1999) considering that they could be useful only in the concrete case of comparative study of two teeth.

The parameters not adopted in the present work are the following:

- Length of the adapical opening. This character revealed to be inconsistent, because it changes within a same tooth according to whether the tooth is dry, as that employed for the SEM photographs) or wet, as in the living animal.

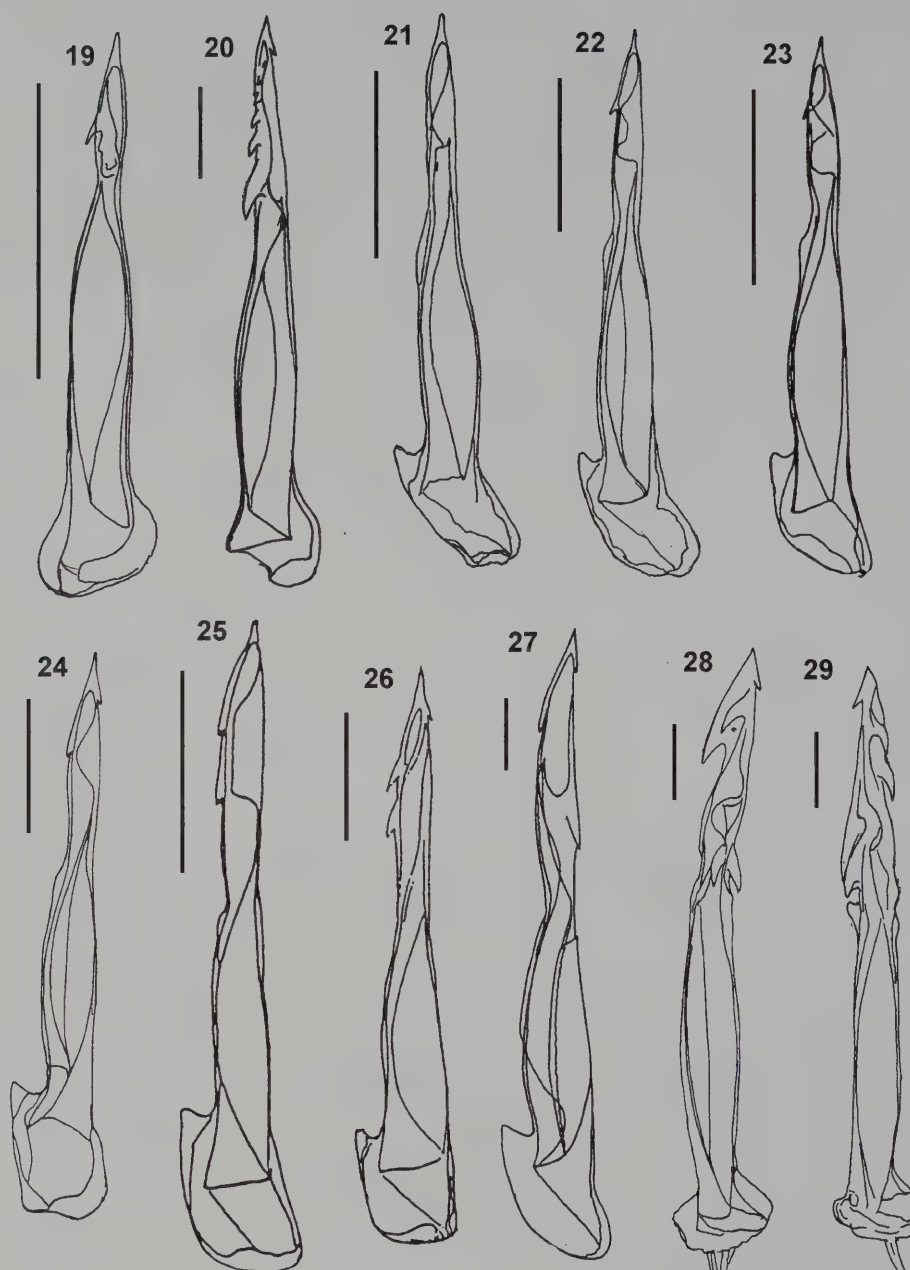
- Length of the serration. This is better represented by the number of the denticles within the serration, the number of denticle rows, and the characteristic of the D.

- Relative barbs length, which is a parameter hardly useful for general comparison.

Finally, we discriminated quantitative and qualitative characters.

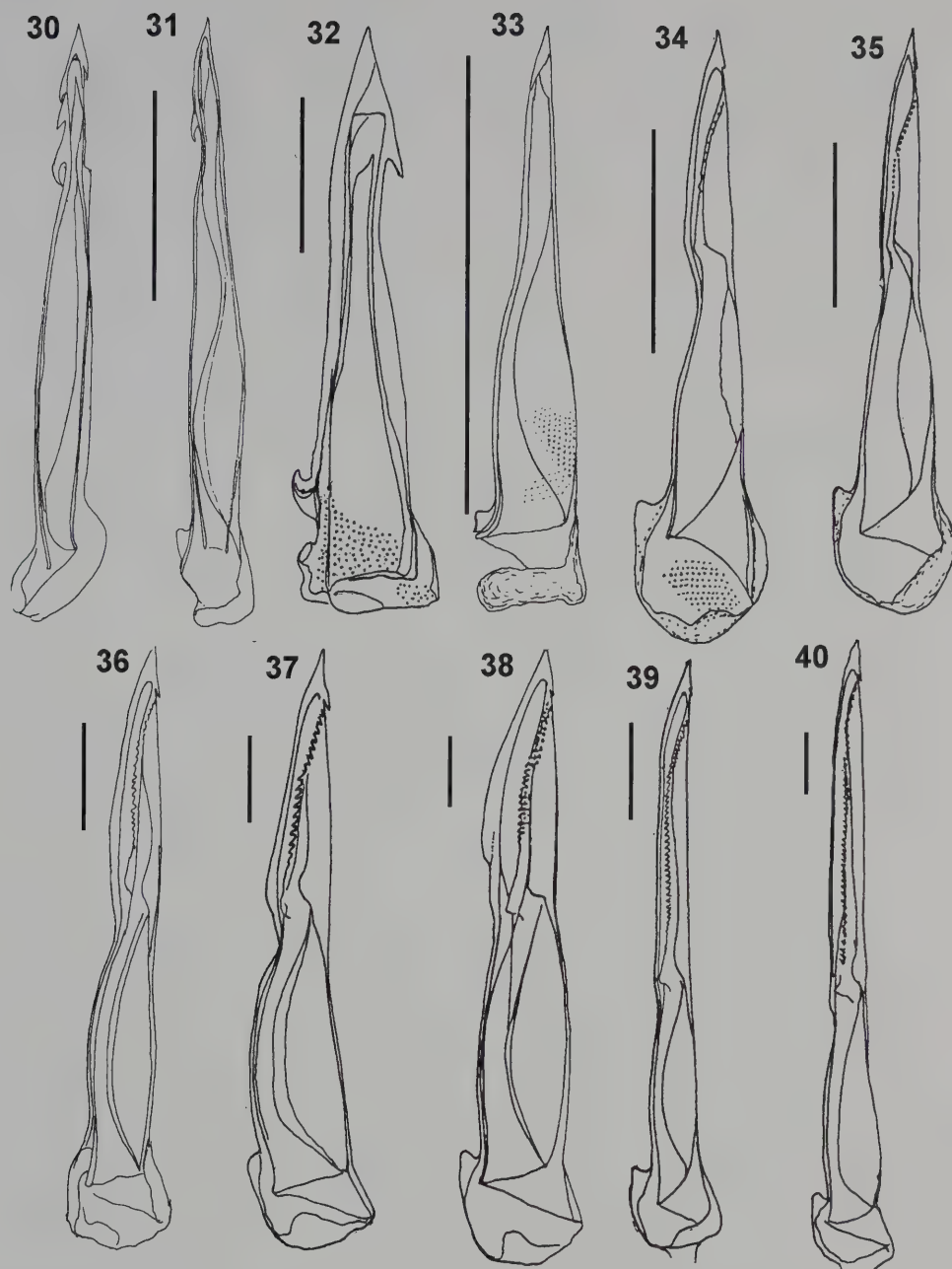
RESULTS

A preliminary analysis of our wide assemblage of data allowed to recognize five main groups in which the radular teeth of *Conus* are distributed.

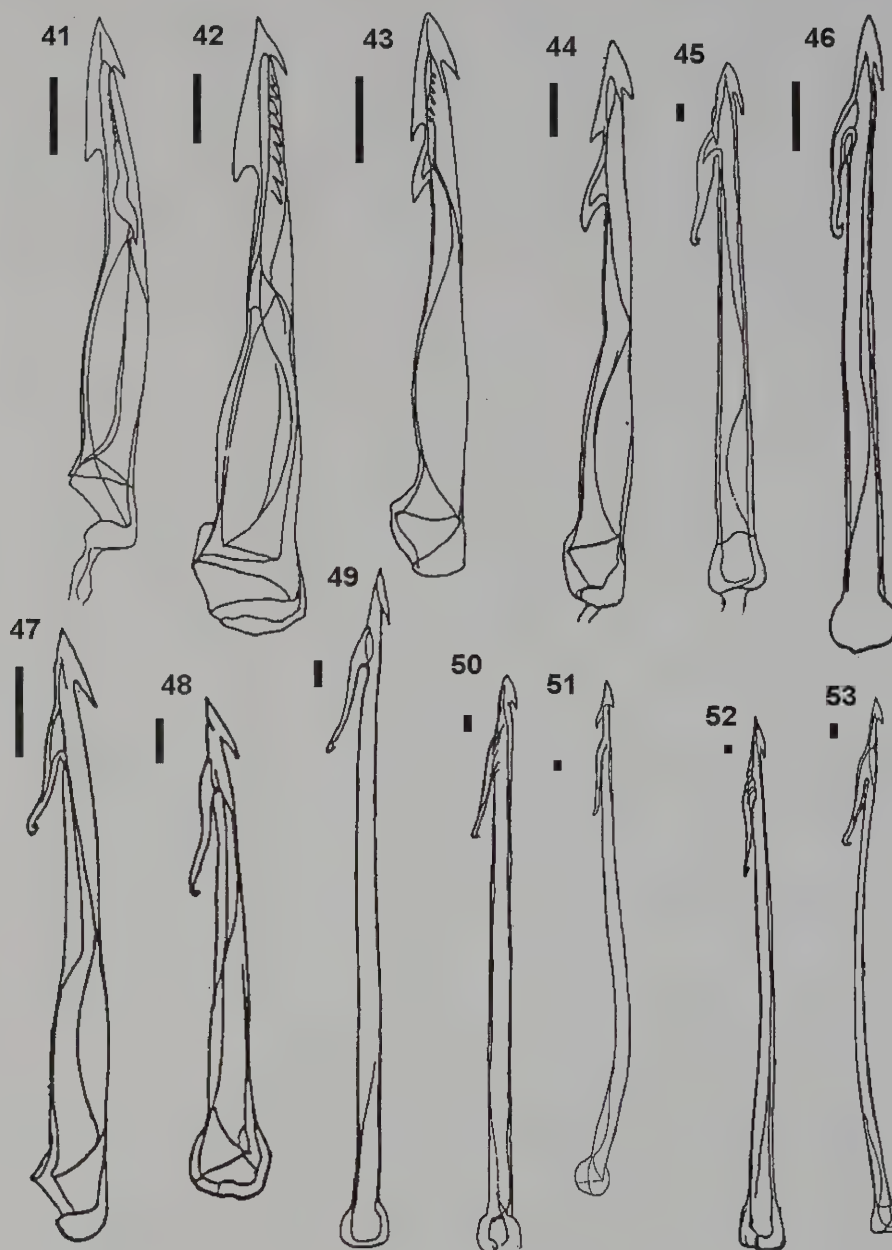
We could have coded them by a letter or by an ordinal number, but we prefer to adopt a more descriptive term since the correlation of radular tooth morphology with feeding habits in *Conus* is perhaps one of the few widely accepted concepts concerning *Conus* biology. Exceptions to this correlation do exist however, with the most striking example being represented by *C. geographus*, a well- known fish hunting species, whose radular tooth is typical of molluscovorous species. The "net strategy" of prey capture adopted by *C. geographus* (Olivera, 1997) may help explaining how adaptive or behavioural traits can superimpose to evolutionary traits. Thus, as already explained in ROLAN & RAYBAUDI MASSILIA (1994a, 1994b) besides the terms "vermivorous", "molluscivorous" and "piscivorous" morphological type of radular tooth, we refer to the "generalist" type, because several deep water species for which the prey is still unknown have

Figs. 19-29. Radular tooth of some Conus species. Scale bar 0.1 mm. Fig. 19. *Conus acutangulus*, shell length 10.0 mm, Hawaii. Fig. 20. *Conus praecellens*, shell length 41.8 mm, Cebu, Philippines. Fig. 21. *Conus jaspideus*, shell length 21.5 mm, Bahamas. Fig. 22. *Conus mindanus*, shell length 26.3 mm, Brazil. Fig. 23. *Conus pealii*, shell length 19.6 mm, Caribbean. Fig. 24. *Conus bozzettii*, shell length 41.8 mm, Cape Ras Hafun, E. Somalia (from Rolán & Raybaudi Massilia, 1994b). Fig. 25. *Conus orbignyi*, shell length 38.0 mm, Philippines (from Rolán & Raybaudi Massilia, 1994b). Fig. 26. *Conus comatosa*, shell length 34.0 mm, Philippines (from Rolán & Raybaudi Massilia, 1994a). Fig. 27. *Conus teramachii*, shell length 67.5 mm, Philippines (from Rolán & Raybaudi Massilia, 1994b).. Figs. 28-29. *Conus californicus*, shell length 28.5 mm, Gulf of California, USA.

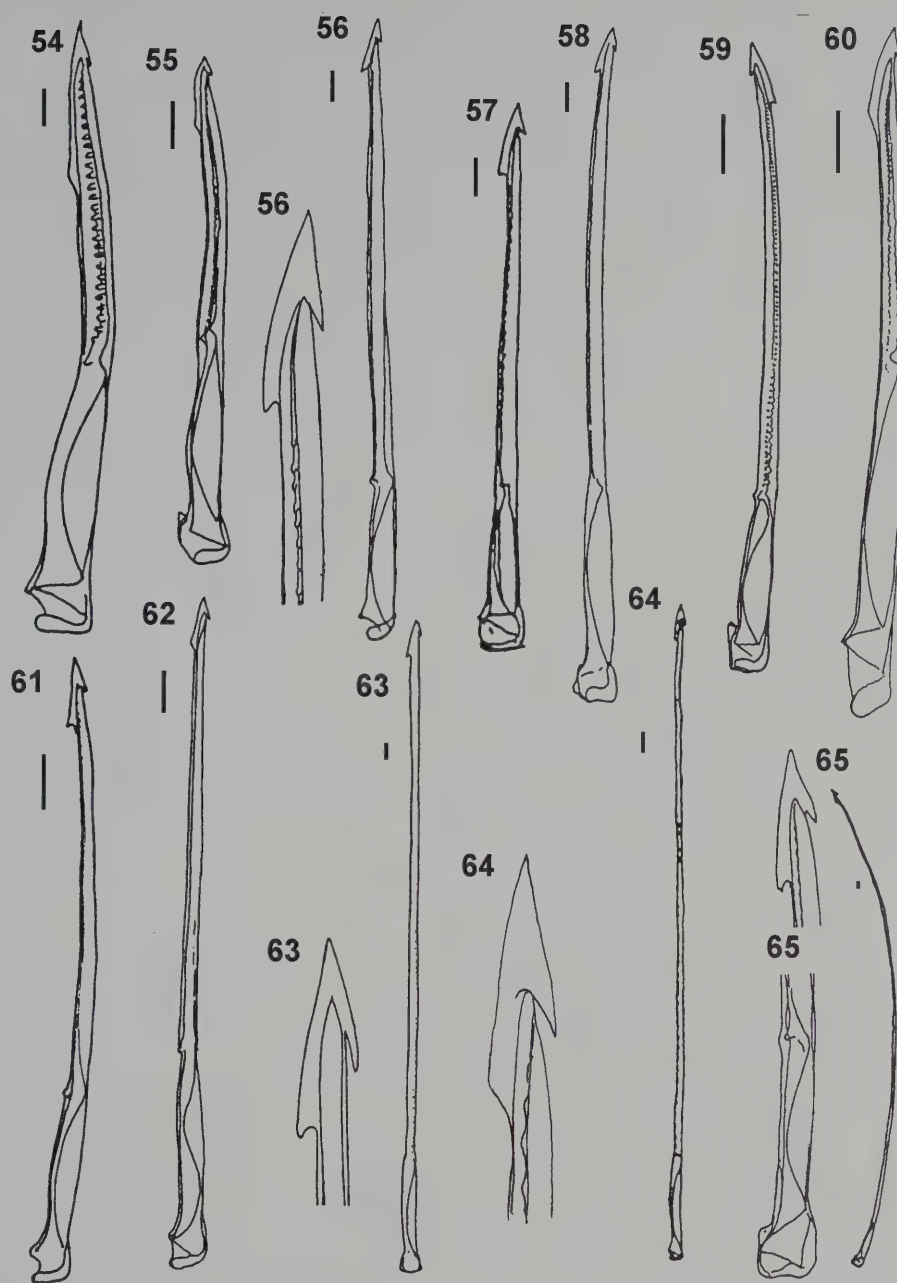
teeth similar to *C. californicus*, a well known generalist feeder. The definition "primitive" type of tooth, though on a less concrete ground, may also refer to generalist or vermivore feeders. It has been chosen because this type of tooth reflects the least derived state of the main characters of the radular tooth of *Conus* compared with turrids radular teeth.

Types of teeth

1- Primitive type of tooth: simple, small teeth (lowest relative tooth length) with few characters (absence of barbs or presence of a single barb, a long saw without a serration, a broad and strongly reinforced base, a prominent basal spur and a poorly defined waist). Teeth with the closest similarity to those of

Figures 30-40. Radular tooth of some Conidae and Turridae species. Scale bar 0.1 mm. Figs. 30-31. *Conorbis coromandelicus*, shell length 37.9 mm, Cuddalore, India. Fig. 32. *Genota marchadi*, shell length 34.5 mm, Dakar, Senegal. Fig. 33. *Genota vafra*, shell length 30.0 mm, Farol das Lagosta, Angola. Fig. 34. *Conus naranjus*, shell length 19.1 mm, Angola (from Rolán & Röckel, 1999) Fig. 35. *Conus flavusalbus* shell length 21.9 mm, Baia das Pipas, Angola (from Rolán & Röckel, 1999). Fig. 36. *Conus ventricosus*, shell length 30 mm, Algarve, Portugal. Fig. 37. *Conus miliaris*, shell length 28.7 mm, Queensland, Australia (from Rolán & Raybaudi Massilia, 1994a). Fig. 38. *Conus borgesi*, shell length 26.3 mm, Baia das Gatas, Cape Verde Is. (from Rolán, 1992). Fig. 39. *Conus franciscoi*, shell length 34.4 mm, Chapeu Armado, Angola (from Rolán & Röckel, 1999). Fig. 40. *Conus guinaicus*, shell length 35.3 mm, Dakar, Senegal.

some turrids (Figs. 1-12). Selected examples include *C. trovaoi* (Fig. 13), *C. neoguttatus* (Fig. 14), *C. naranjus* in early post-metamorphic stage (Fig. 15), but also the adult stages of *C. elegans* (Fig. 16), *C. stocki* (Fig. 17), *C. lizarum* (Fig. 18).

2- Generalist type of tooth: more complex than previous teeth, still very short compared to shell length, with several barbs, a well evident waist and an usually obliquely elongated large base. The adapical opening is still wide and a serration is often just sketched. The first group includes *C. acutangulus* (Fig. 19), *C. praecellens* (Fig. 20), *C. jaspideus* (Fig. 21), *C. mindanus* (Fig. 22), *C. pealii* (Fig. 23) and *C. bozzettii* (Fig. 24); A second group includes *C. orbignyi* (Fig. 25), *C. comatosa* (Fig. 26) and *C. teremachii* (Fig. 27) which can be compared with those of *C. californicus* (Figs. 28-29), *Conorbis coromandelicus* (Figs. 30-31), *Genota marchadi* (Fig. 32) and *Genota vafra* (Fig. 33).

Figures 41-53. Radular tooth of some Conus species (from Rolán & Raybaudi Massilia, 1994a, b). Scale bar 0.1 mm. Fig. 41. *Conus friedae*, shell length 40.2 mm, Sri Lanka. Fig. 42. *Conus cordigera*, shell length 35.5 mm, Indonesia. Fig. 43. *Conus salzmanni*, shell length 18.7 mm, Gulf of Aden. Fig. 44. *Conus jickeli*, shell length 39.7 mm, Gulf of Aden. Fig. 45. *Conus julii*, shell length 52.0 mm, Mauritius. Fig. 46. *Conus solomonensis*, shell length 30.0 mm, Guadacanal, Solomon I. Fig. 47. *Conus zapatosensis*, shell length 19.2 mm, Philippines. Fig. 48. *Conus scalptus*, shell length 22.3 mm, Philippines. Fig. 49. *Conus timorensis*, shell length 35.9 mm, Mauritius. Fig. 50. *Conus achatinus*, shell length 47.7 mm, Thailand. Fig. 51. *Conus ermineus*, shell length 60.1 mm, Cape Verde Is. Fig. 52. *Conus striatus*, shell length 83.0 mm, Philippines. Fig. 53. *Conus leehmani*, shell length 55.3 mm, Maldive Is.

3- Vermivorous type of tooth. This is the most frequently observed type of tooth in our analysis: a medium sized tooth (relative tooth length: LC/DR between 27, in the largest teeth, and 120, in the case of the smallest teeth. These numbers can show us the % of the shell length with the ratio 100/(LC:DR). So, in the mentioned cases the extremes represent 3.6% of the shell length, in the largest teeth, up to 0.83%, in the smallest). The width of the tooth (DR/APA) is in the range 8 – 19. Teeth have usually a well defined waist, a single barb opposing a medium sized blade, a denticulate saw (serration), a moderate central cusp and a more or less conspicuous base with a projecting spur. A typical vermivorous tooth is found chiefly in very shallow or moderately shallow water species. Examples of this type are represented by the tooth of adult specimens of *C. naranjus* (Fig. 34) and *C. flavusalbus* (Fig. 35), as well as by less complex teeth with a lower number of denticles (D) within the serration (S); medium sized vermivorous teeth are also those of

Figures 54-65. Radular tooth of some Conus species (from Rolán & Raybaudi Massilia, 1994a, b). Scale bar 0.1 mm. Fig. 54. *Conus carnalis*, shell length 63.0 mm, Angola. Fig. 55. *Conus algoensis simplex*, shell length 55.5 mm, South Africa. Fig. 56. *Conus rubropennatus*, shell length 46.7 mm, Reunion I. Fig. 57. *Conus amadis*, shell length 48.6 mm, India. Fig. 58. *Conus episcopatus*, shell length 34.3 mm, Thailand. Fig. 59. *Conus terebra*, shell length 40.8 mm, Mauritius. Fig. 60. *Conus moreleti*, shell length 50.5 mm, Hawaii. Fig. 61. *Conus pennaceus*, shell length 21.5 mm, Mauritius. Fig. 62. *Conus lischkeanus*, shell length 57.8 mm, Japan. Fig. 63. *Conus paulucciae*, shell length 55.0 mm, Mauritius. Fig. 64. *Conus ammiralis*, shell length 47 mm, Philippines. Fig. 65. *Conus ammiralis pseudocedonulli*, shell length 65.0 mm, Reunión.

C. ventricosus (Fig. 36) and *C. miliaris* (Fig. 37). Larger teeth are those of *C. borgesi* (Fig. 38), which are broad and with several rows of denticles within the serration, while in other species they are elongate as in *C. franciscoi* (Fig. 39) or *C. guinaicus* (Fig. 40).

4- Piscivorous type of tooth. This is a large and elongate tooth (LC/DR between 30 – 8, that is 3.3% to 12.5 % shell length). The width of tooth (DR/APA) ranges from 21 for the wider teeth, to 120 in the largest. These teeth have three barbs B1, B2, B3: showing different directional arrangement in postmetamorphic transitional stages of development, before attaining reproductive maturity, as in the fully adult stage of *C. friedae* (Fig. 41), *C. cordigera* (Fig. 42), *C. salzmanni* (Fig. 43), and *C. jickeli* (Fig. 44); Barbs may be alternately oriented as in the mature stage of *C. julii* (Fig. 45), *C. solomonensis* (Fig. 46), *C. zapatosensis* (Fig. 47), *C. scalptus* (Fig. 48), *C. timorensis* (Fig. 49), *C. achati-*

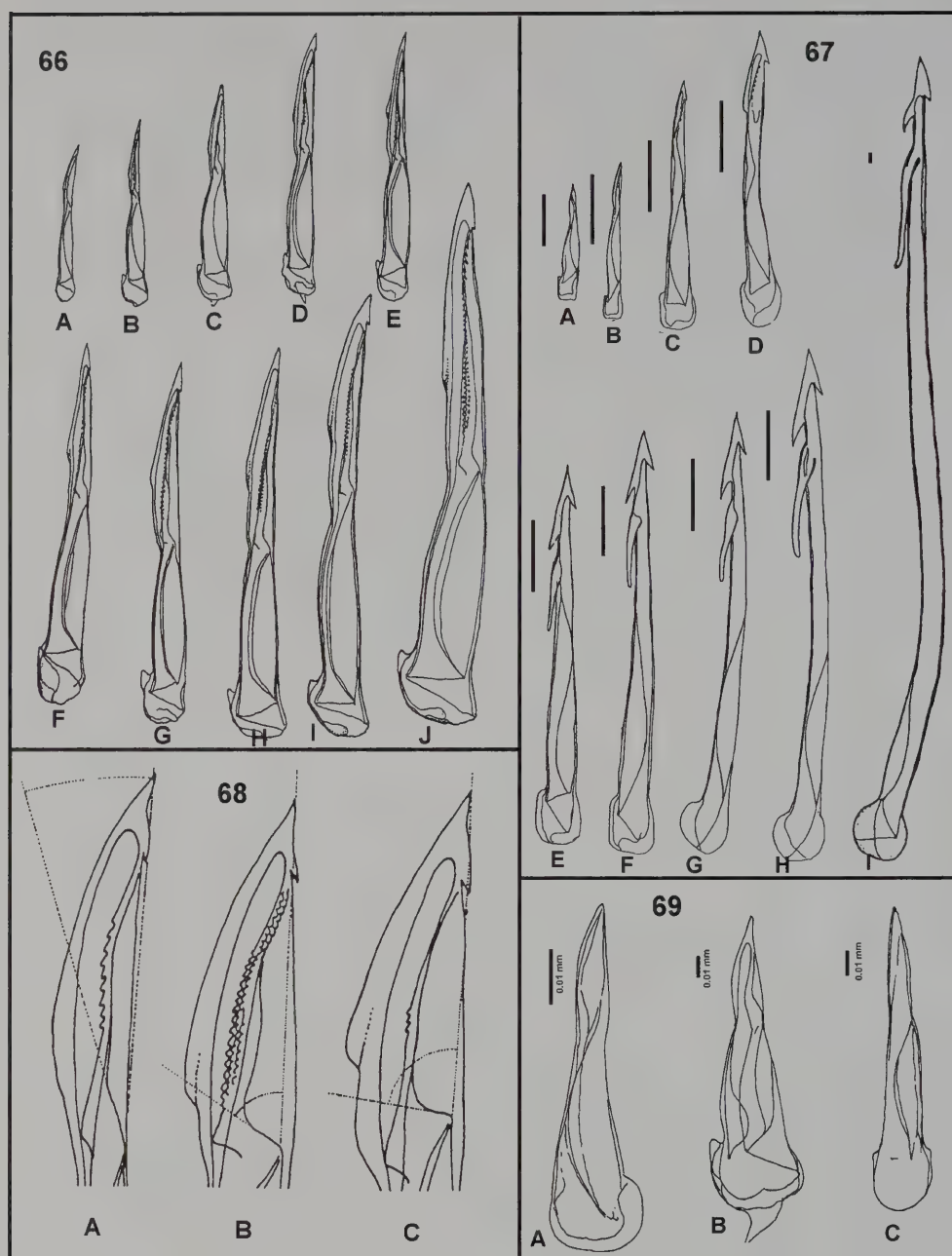



Fig. 66. Growth series of radular teeth from *C. trochulus*, Cape Verde Islands. Shell size of the specimens: A: 14.3 mm. B: 16.8 mm. C: 15.7 mm. D: 17.7 mm. E: 20.1 mm. F: 26.4 mm. G: 31.6 mm. H: 35.3 mm. I: 43.5 mm. J: 52.5 mm. **Fig. 67.** Growth series of radular teeth from *C. ermineus*. Shell size of the specimens: A: 8.0 mm. B: 10.1 mm. C: 13.0 mm. D: 13.0 mm. E: 13.9 mm. F: 12.1 mm. G: 13.4 mm. H: 11.9 mm. I: 60.1 mm. **Fig. 68.** Basal angle of the serration (ABS): A: *C. miruchae*; B: *C. borgesi*; C: *C. navarroi*. **Fig. 69.** Postmetamorphic radular teeth from: A- *C. trochulus*, *C. diminutus* and *C. curralensis*, Cape Verde Is. Scale bar 0.01 mm.

nus (Fig. 50), and teeth may be more elongate as in *C. ermineus* (Fig. 51), C. *striatus* (Fig. 52) and *C. leehmani* (Fig. 53). Here again, three subgroups may be further splitted upon qualitative (i.e. orientation of barbs) and quantitative parameters.

5- Molluscivorous type of tooth. They are among the largest and narrowest teeth observed (LC/DR ranging from 27 up to 9, corresponding to a relative tooth length between 3.7 and 11% LC). Teeth are very narrow, with DR/APA ranging from 15 to 100 in the most elongate teeth. These teeth have a blade replacing Barb 2, as it is observed in the ontogeny of some species as *C. carnalis* (Fig. 54), *C. algoensis simplex* (Fig. 55) or in fully grown individuals as in *C. rubropennatus* (Fig. 56), *C. amadis* (Fig. 57), *C. episcopatus* (Fig. 58), *C. terebra* (Fig. 59), *C. moreleti* (Fig. 60), *C. pennaceus* (Fig. 61), *C. lischkeanus* (Fig. 62), *C. paulucciae* (Fig. 63), *C. ammiralis* (Fig. 64) and *C. ammiralis pseudocedonulli* (Fig. 65). Some subgroups may be further defined within this type of tooth.

Quantitative characters

The characters which will be commented here are those usually

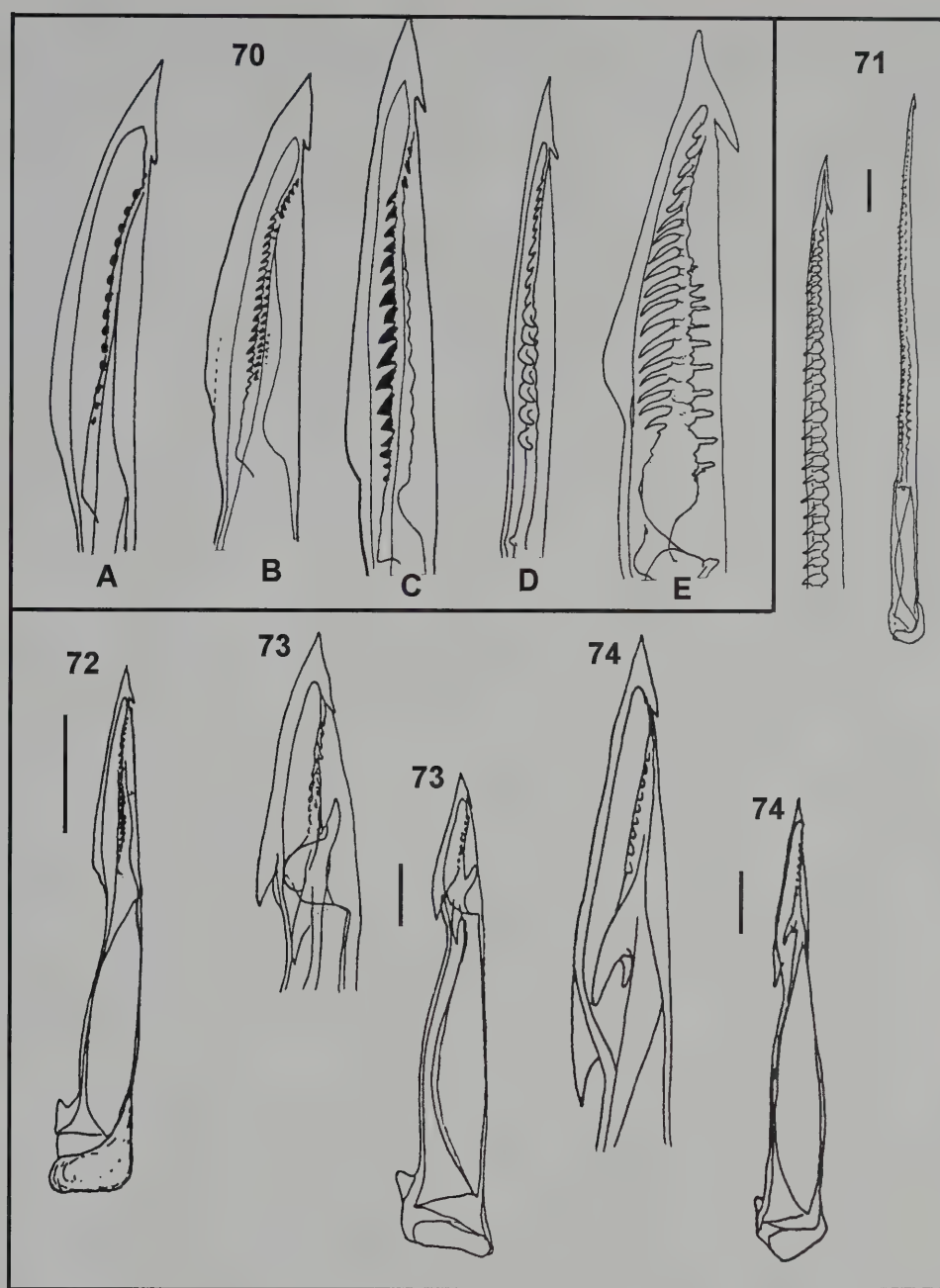



Fig. 70. Shape of the denticles within the serration of the radular tooth: A: *C. miruchae*. B: *C. ateralbus*. C: *C. tabidus*. D: *C. planorbis*. E: *C. miles*. Scale bar 0.1 mm. **Fig. 71.** *C. splendidulus*, shell length 51.0 mm, Little Aden **Fig. 72.** *C. characteristicus*, shell length 17 mm, Thailand **Fig. 73.** *C. duffyi*, shell length 35.0 mm, Los Roques, Venezuela **Fig. 74.** *C. imperialis*, shell length 36.0 mm, Reunion.

employed in the radular studies and illustrated in previous works, for instance, in ROLÁN (1992).

1 Number of teeth within the radula sac (ND)
Counting the total teeth within the radula sac is easy if the animal has been preserved in alcohol and the entire content of radular sac can be studied. This information is not easy to get in those cases in which the radula is studied from dry animals. Though this information is not available for all the species we have examined, the sample is wide enough to allow generalization. This character may be not independent from relative tooth length and a correlating test should be carried out before use for statistical purposes, however we selected it because it may well allow prediction of prey type and envenomation strategy.

For turrids, we have not such a wide body of information on radular teeth, however we could observe that in some species only 7 teeth were present in the radula sac, as in *Mangelia angolensis*; usually the number of teeth was between 26 in *Genota vafra* up to more than 100 teeth, as it observed in some species of *Crassispira* (see FERNANDES, ROLÁN & OTERO-SCHMITT, 1995)

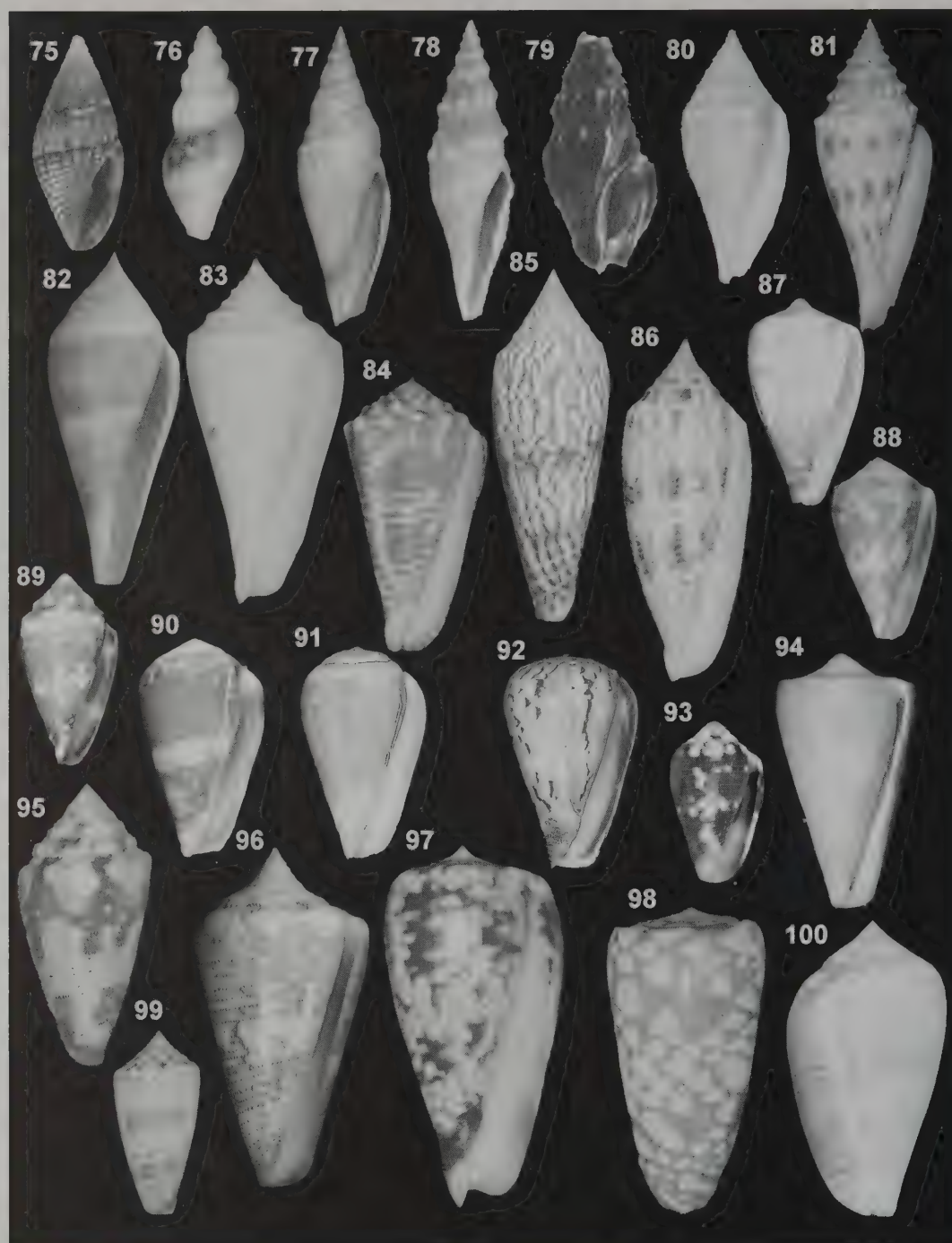



Fig. 75. *Mitrolumna monodi*, 4.4 mm, Cap Vert, Senegal (from Rolán & Boyer, 2000) **Fig. 76.** *Mangelia albilonga*, 8.0 mm, paratype (CER), Buraco, Palmeirinhas, Angola **Fig. 77.** *Genota marchadi*, 34.3 mm, Dakar, Senegal (from Rolán & Raybaudi, 1994b) **Fig. 78.** *Genota mitraeformis*, 49 mm, Gabon (from Rolán & Raybaudi, 1994b) **Fig. 79.** *Crassispira funebralis*, 32 mm, Pointe Noire, Congo **Fig. 80.** *Conorbis coromandelicus*, 40 mm, Coromandel Coast, India (from Röckel, Korn & Kohn, 1995, courtesy ConchBooks, Germany) **Fig. 81.** *Conus orbignyi elokisimenos*, 60 mm, Natal, South Africa (from Rolán & Raybaudi, 1994b) **Fig. 82.** *C. profundorus*, 113 mm, Balut Islands, Philippines (from Rolán & Raybaudi, 1994b) **Fig. 83.** *C. teramachii*, 102.4 mm, Japan (from Rolán & Raybaudi, 1994b) **Fig. 84.** *C. sulcocastaneus*, 51 mm, Punta Engaño, Philippines (from Rolán & Raybaudi, 1994b) **Fig. 85.** *C. ranonganus*, 100 mm, Ranong, Thailand (from Rolán & Raybaudi, 1994b) **Fig. 86.** *C. australis*, 85.7 mm, Taiwan (from Rolán & Raybaudi, 1994b) **Fig. 87.** *C. borgesi*, 26.1 mm, holotype (MNCN), Gatas, Boa Vista, Cape Verde Archipelago **Fig. 88.** *C. curralensis*, 24.8 mm, holotype (MNCN), Curral, Santa Luzia, Cape Verde Archipelago **Fig. 89.** *C. navarroi*, 19 mm, holotype (MNCN), Calhau, São Vicente, Cape Verde Archipelago **Fig. 90.** *C. babaensis*, 25.8 mm, holotype (MNCN), Baia da Baba, Angola (from Rolán & Röckel, 2001) **Fig. 91.** *C. flavusalbus*, 23.7 mm, holotype (MNCN), Baia das Pipas, Angola (from Rolán & Röckel, 2001) **Fig. 92.** *C. trovaoi*, 38 mm, holotype (MNCN), Limagens, Angola (from Rolán & Röckel, 2001) **Fig. 93.** *C. hieroglyphus*, 14 mm, Aruba, Antilles **Fig. 94.** *C. daucus*, 25.5 mm, Los Canarreos, Cuba **Fig. 95.** *C. duffyi*, 40.5 mm, Los Roques Archipelago, Venezuela (from Rolán & Raybaudi, 1994a) **Fig. 96.** *C. cedonulli*, 44.0 mm, St. Vicente, Antilles **Fig. 97.** *C. spectrum*, 69 mm, Gladstone, Queensland, Australia (from Rolán & Raybaudi, 1994b) **Fig. 98.** *C. cordigera*, 40.0 mm, Balabac, Palawan, Philippines (from Rolán & Raybaudi, 1994b) **Fig. 99.** *C. satzmanni*, 27 mm, Little Aden (from Rolán & Raybaudi, 1994b) **Fig. 100.** *C. scalptus*, 28 mm, syntypes (BMNH), locality unknown (from Rolán & Raybaudi, 1994b)

and up to 134 teeth in the radula sac of *Mitrolumna monodi* (see ROLÁN & BOYER, 2001). The primitive type of tooth is generally present in a higher number within the radula sac of vermivorous and generalist species of *Conus*. On the contrary, the molluscivorous and piscivorous types of tooth are usually present in lower number. The lowest observed number of teeth in radula sac was 20 for *Conus regius*, a specialized Amphinomids- hunting species. The highest ND was 130, observed in *C. beilarensis*. Thus, the general trend appear to have been towards a decrease of number, together with an increase of dimensions of the tooth.

Table 1 resumes the state of this character in a selected sample of species for each group.

Therefore, we conclude that a high number of teeth within the radular sac, a shared state of the character in turrids and in the majority of *Conus* species (generalist and vermivorous species) is plesiomorphic.

Conversely, a low number of teeth within the radula sac, a shared state of the character among the most derived type of teeth, i.e. the molluscivorous and the piscivorous type is apomorphic.

2. Relative length of the radular tooth, calculated as the ratio of shell length to DR (LC/DR)

As mentioned above, this character can be transformed in % of the shell length. This is the most important quantitative character because discrete intervals clearly separate at least the three main large trophic groups of *Conus* species.

Table 2 resumes data for some turrids and *Conus* species (most of data are from ROLÁN & RAYBAUDI (1994a, 1994b).

The general trend towards an increase in absolute as well as relative length of the tooth is evident from our sample.

From the analysis of our wide data set, we conclude that small size of the radular tooth, a state of the character shared by turrids, generalist and vermivorous species of *Conus* is plesiomorphic.

Conversely, the large size of radular tooth attained by fewer, extremely specialized species (including species known also from molecular study to have diverged very recently (i.e. *C. consors-C. magus*, *C. purpurascens-C. ermineus*, ESPIRITU *ET AL.*, 2001), i.e. piscivorous and molluscivorous species represent an apomorphy.

3. Presence (and position) or absence of Waist (W)

Waist has been defined as the constriction of the shaft, the columnar body of the radular tooth (NYBAKKEN, 1970). A waist is absent in old turrids and in many species of higher turrids. When present in higher turrids (Conidae *sensu* TAYLOR *et al.*, 1993) (i.e. *Mitrolumna sp.*, (Figs. 6-7) *Lovellona sp., Genota marchadi* (Fig. 32) it is located at the adapical third.

In *Conus* and *Conorbis*, a waist is present in the primitive and generalist type of tooth (as in *Conorbis coromandelicus*, Figs. 30-31), located at the same high position. During the post-metamorphic developmental stages of most vermivorous species, but often even in intra-capsular stages, a waist is initially present at the adapical third of the tooth reaching a central position at full maturity. The waist disappears in the transitional stages of a piscivorous tooth and is practically absent in the molluscivorous type of teeth.

Figs. 66 and 69A illustrate the presence of the waist during the ontogeny of the worm-hunting *C.trochulus*; Its presence in the generalist type of tooth can be observed in Figs. 19-29 and in Figs. 30-31 (*Conorbis coromandelicus*). Fig. 67 demonstrates that a waist is present only in the earliest ontogenetic stages of the fish-hunting *C. ermineus*.

In higher turrids, the presence of a waist can be observed in

Table 1. Average number of teeth in the radular sac for a selected sample of adult specimens from species differing in trophic specialization and geographic distribution

Conidae	shell size in mm (LC)	number of teeth(ND)
Vermivorous type:		
C. arenatus	26.6	22
C. limpusi	35.2	27
C. papuensis	22.9	30
C. daucus	31.3	30
C. borgesi	34.5	32
C. segravei	29.6	35
C. tribblei	54.3	42
C. ventricosus	32.4	47
C. pineaui	24.4	50
C. eburneus	40.3	57
C. bulbus	18.4	63
C. babaensis	24.8	78
C. zebroides	30.8	102
C. belairensis	32.0	122
piscivorous type		
C. salzmanni	18.7	20
C.ermineus	26.0	24
C. striatus	83.0	24
C. julii	52.0	30
C. terminus	55.2	38
C. achatinus	50.5	42
molluscivorous type		
C. lischkeanus	57.8	31
C. quercinus	107.6	40
C. patonganus	44.0	44
C. paulucciae	49.3	74
generalist type		
C. praecellens	41.8	44
C. profundorum	35.5	48
C. pagodus	48.5	71
C. jaspideus	21.5	85
C. deyncerorum	14.1	87

Table 2. Ratio between shell length (LC) and radular tooth length (DR) in several species of the different radular groups of Conidae, showing the % of tooth length with respect to shell length.

Conidae	LC	DR	100/(LC/DR) = %
vermivorous type			
C. limpusi	32.4	0.66	2.3
C. gondawanensis	18.8	0.36	1.9
C. clarus	31.8	0.50	1.5
C. reductaspiralis	39.8	0.75	1.9
C. ventricosus	29.6	0.61	2.0
C. queenslandis	77.5	0.95	1.2
C. rufimaculosus	36.0	0.80	2.2
C. papuensis	22.9	0.36	1.6
C. wallangra	29.9	0.63	2.1
C. gloriakiensis	64.0	0.83	2.1
C. ritae	18.4	0.50	2.7
C. eburneus	40.3	0.49	1.2
C. pauperculus	16.8	0.37	2.2
C. cuvieri	11.3	0.41	3.6
C. miliaris	10.1	0.24	2.3
C. musicus	19.5	0.45	2.3
C. shikamai	16.7	0.42	2.5
C. characteristicus	25.8	0.58	2.2
C. pulicarius	17.2	0.32	1.8
C. hieroglyphus	18.6	0.39	2.1
C. echinophilus	13.5	0.26	1.9
C. belairensis	32.0	0.31	0.9
C. mercator	32.0	0.77	2.4
C. coffeae	36.5	0.70	1.9
C. fuscolineatus	31.0	0.79	2.5
piscivorous type			
C. sertacinctus	27.6	1.35	4.8
C. scalptus	22.3	1.15	5.1
C. barthelemyi	19.0	1.34	7.0
C. lovellreveei	33.2	1.37	4.1
C. stercusmuscarum	48.9	5.45	11.1
C. solomonensis	30.0	2.50	8.3
C. achatinus	47.7	4.25	8.9
C. mucronatus	26.9	1.11	4.1
C. gubernator	53.7	4.50	8.3
C. monachus	31.8	2.95	9.2
C. julii	52.0	3.00	5.7
C. terminus	55.2	6.12	11.0
C. striatus	62.8	7.20	11.5
molluscivorous type			
C. geographus	67.0	7.80	11.6
C. crocatus	50.2	2.30	4.5
C. paulucciae	55.0	4.55	8.2
C. omaria	57.0	4.60	8.0
C. victoriae	54.0	4.50	8.3
C. lischkeanus	57.8	2.05	3.5
C. barbieri	26.0	1.91	7.3
C. patonganus	44.0	3.35	7.6
C. acuminatus	35.0	1.20	3.4
C. lividus	45.2	1.87	4.1
C. episcopatus	34.3	2.22	6.4
C. terebra	40.8	1.25	3.1
C. pennaceus	21.5	1.24	5.7
C. eximius	47.5	1.85	3.9
C. nicobaricus	50.7	1.03	2.0
C. quercinus	54.5	1.19	2.1
C. marmoreus	53.0	3.00	5.6
C. splendidulus	50.0	1.33	2.6
C. obscurus	28.1	3.85	13.7
generalist type			
C. vaubani	29.3	0.95	3.2
C. loyaltiensis	22.4	0.60	2.6
C. deyncerorum	15.0	0.28	1.8
C. eugrammatus	24.0	0.49	2.0
C. lizarum	20.8	0.25	1.2
C. pagodus	48.5	0.43	0.9
C. kimioi	14.0	0.26	1.8
C. rutilus	11.8	0.44	3.7
C. profundorum	35.5	0.52	1.4
C. aphroditae	16.1	0.25	1.5
C. orbignyi	38.0	0.32	0.8
C. lucidus	27.0	0.33	1.2
C. jaspideus	21.5	0.30	1.4
C. pealii	16.4	0.25	1.5
C. memiae	25.5	0.55	2.1
C. delesserti	60.6	0.61	1.0
C. bozzettii	41.8	0.42	1.0
C. acutangulus	10.0	0.18	1.8
C. praecellens	41.8	0.63	1.5
C. aff. *vanhingi*	16.7	0.30	1.7
C. stocki	26.8	0.25	0.9
C. longurionis	35.5	0.38	1.0
Conorbis coromandelicus	37.9	0.34	0.9
Turridae			
Genota marchadi	34.3	0.38	1.1
Genota vafra	30.0	0.12	0.4
Mitrolumna monodi	4.2	0.08	1.9
Mitrolumna saotomensis	3.7	0.07	1.9
Mangelia merlini	7.0	0.18	2.6

Genota marchadi (Fig. 32) and in *Mitrolumna* (Figs. 6-7) (see ROLÁN & BOYER, 2001).

Though a character certainly more typical of the tightly coiled *Conus* radular tooth than the turrids tooth, the presence of a waist is a shared character of some higher turrids, of the generalist species of *Conus* and *Conorbis* and of the great majority of vermivorous *Conus*.

We conclude that the presence of a waist is a plesiomorphy. The position of the waist may usefully differentiate the generalist type of tooth and several subgroups of vermivorous. Thus, this character would be better represented by a two state character: presence–absence and a binary state for its position: a) Present and high (about 1/3 radular tooth), b) Present and central (about 1/ 2 of the radula tooth) and –Absent (lost)

Thus, absence of a waist is a plesiomorphy in turrids. Loss of a waist is considered a derived state in *Conus*.

4. Relative length of the apical portion (PA), calculated by its relation with the absolute tooth length (DR/PA)

In the studied turrids, when a waist exists, it is located at the apical third (already mentioned for *Genota* and *Mitrolumna*).

All the vermivorous teeth studied in their ontogeny definitively show a short PA which increases in advanced stages. Examples may be found in ROLÁN (1992) (see Fig. 66), and in NYBAKKEN (1990).

In fish-hunting *Conus*, as *C. ermineus*, as well as in all the piscivorous type of teeth examined, PA is observed to increase since the earliest post-metamorphic stages (ROLÁN & BOYER, 2000) (Fig. 67). PA also increases during the ontogeny of the molluscivorous type of tooth, *C. fergusoni*, *C. pennaceus*, (see NYBAKKEN, 1988).

It is necessary to explain that because of the loss of the waist (W) in adult piscivorous and molluscivorous teeth, PA can be calculated only from the PB crossing point with the shaft, which is evident and marks the boundary between PA and PB.

We conclude that a PA <_ DR is a shared state of the character among higher turrids, and of generalist species and vermivorous species in *Conus* and *Conorbis*; additionally the PA increase is well documented by the ontogenetic change observed in worm-hunting, fish- and mollusc-hunting species of *Conus*.

Thus, we consider PA<1/2 DR a plesiomorphic character. Conversely, the extremely elongated PA observed in the molluscivorous type of tooth is here considered the most derived state of the character.

5. Presence or absence of a blade (F)

In turrids, the presence of a structure similar to a blade is infrequent. However the radular tooth of some species of *Benthofascis* (Fig. 12), *Phenatoma*, *Typhlodaphne*, and *Pontiothauma* (see POWELL, 1966) have a well defined blade.

In the tooth of post-metamorphic juvenile specimens of worm-hunting or fish-hunting *Conus*, there is no evidence of a blade in PA, (Figs. 66, 67 and 69). F is absent in the most primitive vermivorous teeth as *C. trovaoi* (Fig. 13), *C. neoguttatus* (Fig. 14) and *C. naranjus* (Fig. 15).

In mature stages of the vermivorous type of *Conus* tooth, the blade is usually covering most of PA, as it may be observed in *C. ventricosus* (Fig. 36), *C. miliaris* (Fig. 37), *C. borgesi* (Fig. 38). F is variable in size, as can be observed in *C. miruchae*, *C. borgesi* and *C. navarroi* (Fig. 68) or in other species (Fig. 70).

Thus, the presence of a blade is considered a derived state shared by only some turrids and by *Conus*.

6 Relative length of the blade (F) (as % of PA)

In a large number of *Conus* where a short F has been observed in the adult stage, a larger F has been observed during juvenile stages. Examples of the ontogenetic change of F are represented in Fig.66 for *C. trochulus*, a worm-hunting species.

A blade is probably present also in the generalist tooth; however, in this group the blade appears to turn into a barb (B) very rapidly during the ontogeny, therefore it is difficult to observe its transition (Figs. 19-27).

In the molluscivorous type of tooth, F is extremely short and it decreases inversely with tooth length (Figs. 54-65).

In fish-hunting species adopting the hook-and line capture strategy (Olivera, 1997)as in *C. ermineus* (Fig. 67) the gradual shortening of F may be observed during the transition from the vermivorous to the piscivorous tooth, a transition which has been correlated with a switch in the targeted prey from polichaets to fish. During the transitional stages the blade is shorter either in relation to PA and DR.

In the piscivorous tooth of fully adult, fish-hunting individuals, the blade is definitively turned into a barb (B2). Thus, in these teeth a blade is not absent but "shortened" since it has been transformed in an additional barb.

In conclusion: a high value of relative length of the blade is a primitive state of the character in the vermivorous tooth, while a short F is a derived state of the character. Finally, a very short blade changed into a second barb during the transitional stages from the vermivorous to the piscivorous type of tooth demonstrates that a short or transformed F is an apomorphic character shared by species of *Conus* possessing piscivorous and molluscivorous types of tooth. This situation is probably better described by a three.states of the characters.

7. Presence or absence of denticles in serration (D)

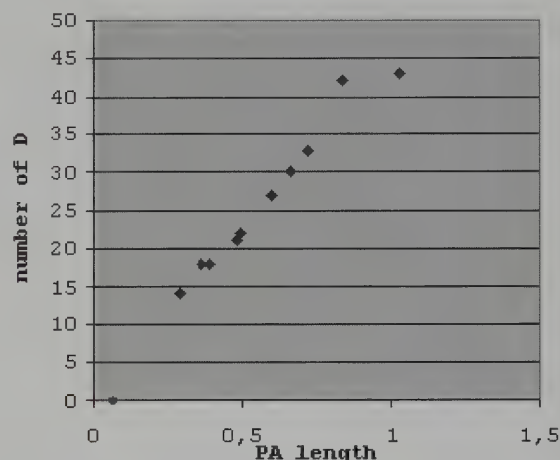
Serration is defined as a longitudinal row of denticles extending along or proximally from the adapical opening of the lumen (LOVEN,1847; PEILE, 1939; ROLÁN, 1992; KOHN *et al.*, 1999). When present, one or more rows of denticles may be present

A serration is absent in the radular teeth of almost all the species of the higher turrids examined. An apparently single row of small, rounded denticles was observed only in some species of *Mangelia* (Figs. 3, 10-11) but the interpretation of this imagine is dubious, because they could be nodules or holes.

In *Conus*, a serration is present in the mature tooth of one group of generalist species, as *C. acutangulus* (Fig. 19) and *C. praecellens* (Fig. 20), where it consists only of three denticles, early transformed into cusps during the ontogeny. In the primitive type of teeth and during the juvenile stages of the vermivorous teeth, denticles are either absent, as in *C. trochulus*,

Table III A.

Number of denticles in serration (D) (counting only a single row) during the ontogeny of *C. trochulus* from Cape Verde Islands.

Shell	LC in mm	DR	D in S
Fig 69 A	1.39	0.06	0
Fig. 66 A:	14.3	0.29	14
B:	16.8	0.36	18
C:	15.7	0.39	18
D:	17.7	0.48	21
E:	20.1	0.49	22
F:	26.4	0-62	27
G:	31.6	0.66	30
H:	35.3	0.72	33
I:	43.5	0.84	42
J:	52.5	1.03	43

C. diminutus and *C. curralensis* (Fig. 69), or they are just sketched and rounded in the most simple teeth, as in *C. naranjus* (Fig. 34) or in *C. fuscoflavus* (Fig. 35). Adult individuals of the great majority of worm-hunting *Conus* species have one or more rows of denticles within the radula serration.

Denticles are absent in the early post-metamorphic vermivorous stages of the piscivorous *C. ermineus*; they are present in the transitional stages; then again they are absent i.e. lost completely) in the mature piscivorous tooth (Fig. 67). D are visible in species which retain as adult, an intermediate form of the piscivorous type of tooth, as in *C. cordigera* (Fig. 42) and *C. salzmanni* (Fig. 43).

In the molluscivorous type of tooth the serration is vestigial and the small and rounded denticles are likely non-functional (Figs. 57-60, 64).

We conclude that absence of a serration is a primitive state of the character shared by most turrids and by *Conus* species, during the earlier developmental stages of the generalist, primitive and vermivorous type of tooth The presence of denticles within the serration is here considered a derived state of the character shared by the majority of worm-hunting species of *Conus*. The vestigial presence of a serration in the mollusc-hunting species and the complete loss of D in fish-hunting species are considered as the most derived state of the character.

8 Number of denticles in serration (D)

In the generalist type of *Conus* teeth in which a serration is present, the number of denticles is very low (3-5) (Figs 19-20). In the primitive type of tooth, denticles were observed sometimes, in teeth clearly evolving towards a molluscivorous type, as in *C. carnalis* (Fig. 54) or *C. splendidulus* (Fig. 71).

The ontogeny of a typical worm-hunting species as (for example *C. trochulus*, Fig. 69) shows that in early post-metamorphic stages the saw bears no denticles, or they are few and arranged in a single row, while in the following stages an appreciable number of D increases with shell length. This was similar for other species studied.

The vestigial denticles in the hidden, non-functional serration of the larger molluscivorous teeth are scantly visible, nevertheless their number is enormously increased: the serration of *C. ammiralis pseudocedonulli* (a mollusc-hunting species) (Fig. 65) bears more than 300 denticles. Moreover, the number of denticles correlates directly with an increasing PA, as can be observed in Table III, for *C. trochulus*.

From our results, we conclude that a low number of D is a plesiomorphy shared by a group of *Conus* species with a generalist and primitive type of tooth and by subadult individuals of almost all the worm-hunting *Conus* species examined.

Conversely, the higher number of D shared by adult worm-hunting and mollusc-hunting species of *Conus* is considered a derived character. The loss of a serration is considered an apomorphy peculiar of the adult piscivorous tooth.

9. Number of rows of D

This character is not present in each type of tooth. However, since it is so widely distributed in the main trophic group (worm-hunting species probably approaches >70% of *Conus* species) it is useful to analyse its state for differentiating sub-

Table IV. Relation between PA (relative length in mm) and D in several species.

C. barbieri	26.0	40
C. crocatus	50.2	60
C. lischkeanus	57.8	31
C. lividus	45.2	50
C. acuminatus	35.0	48
C. patonganus	44.0	44
C. paulucciae	49.3	74
C. pennaceus	21.5	43
C. quercinus	39.0	90
C. quercinus	107.6	40
C. terebra	40.8	52
C. victoriae	54.0	50

groups of species with a vermivorous type of tooth).

The ontogenetic change observed in several species (*C. borgesi*, *C. trochulus* in ROLÁN, 1992) show a clear trend towards an increase in number of denticles within a single row, as well as an increase in number of rows.

We thus conclude that, within the vermivorous teeth of *Conus*, a single row of denticles within the serration is a primitive state of the character, while two or more rows represent a derived state.

Qualitative characters

10. Shape of denticles within the serration

This character is not present in turrids and in some generalist teeth, but in at least one large group of species with a generalist type of tooth (*i.e.* species usually assigned to (sub) genus *Conasprella* Iredale) the few denticles D are highly enlarged like in *C. praecellens* (Fig. 20).

In the vermivorous type of tooth and during the developmental stages of post-metamorphic molluscivorous and/or piscivorous teeth, the early appearing denticles in the serration are like small tubercles, as in *C. miruchae* (Fig. 70 A). In most species or in more advanced developmental stages, the denticles often become sharp-pointed, as in *C. ateralbus* (Fig. 70 B) and increase their size, sometimes very evidently, as in *C. tabidus* (Fig. 70 C), sometimes curving, as in *C. planorbis* (Fig. 70 D) and sometimes becoming very elongated as in *C. miles* (Fig. 70 E).

Tubercle-like denticles are thus considered a primitive state of the character, while elongated or pointed is a derived state of the character.

11. Basal angle of the serration (ABS)

The ABS is the angle formed by the basal part of the serration, in the side opposite to the cusp, with the axis of the tooth (Fig. 68) (ROLÁN, 1992 and ROLÁN & RAYBAUDI MASSILIA, 1994a).

In turrids, it is not easy to find a serration and to measure its angle with the tooth axis. In some teeth as in *Genota marchadi* (Fig. 32) a very acute angle can be supposed.

This character is difficult to be interpreted in some generalist teeth as in *C. teremachii* (Fig. 27) or in *C. californicus* (Figs. 28-29). At least in one group of generalist teeth where the few denticles D are highly enlarged as, for example, in *C. acutangulus* (Fig. 19) or in *C. praecellens* (Fig. 20), the ABS can be studied and it is acute. In other teeth, even without a serration, where the ABS can be supposed, as in *C. elegans* (Fig. 16), *C. stocki* (Fig. 17) or *C. lizarum* (Fig. 18) the angle is acute.

As it may be observed in *C. naranjus* (Figs. 15) and in *C. lizarum* (Fig. 18), the is ABS is acute in the primitive, older vermivorous and even in the more derived type of tooth of *C. miruchae* (Fig. 68 A).

In the most derived vermivorous teeth the ABS can reach 60°, like in *C. borgesi* (Fig. 68 B) or near 90° as in *C. navarroi* (Fig. 68 C). This angle increases in the intermediate type of molluscivorous tooth as in *C. carnalis* (Fig. 54) or in *C. moreleti* (Fig. 60). However, this angle can not be evaluated in the larger molluscivorous and piscivorous teeth perhaps because elongation of the teeth makes it very acute.

Thus, in the vermivorous type of tooth an acute ABS is plesiomorphic and increase of the angle is the derived state. The state of the character is less clear in the primitive, generalist or in the molluscivorous and piscivorous type of teeth.

12. Presence or absence of a cusp (C) and its size

A cusp is absent in all the turrid radular teeth known.

In the earlier developmental stages of the post-metamorphic vermivorous type of tooth there is no prominence at the base of the S, (for example in *C. elegans* Fig. 16). In several generalist

Table V. Polarity state of the radular tooth characters studied.

	PLESIOMORPHIC	APOMORPHIC
Number of teeth within the radula sac (ND)	High number of teeth	Low number of teeth
Relative length of the radular tooth (LC/DR)	Small tooth	Large tooth
	High value of ratio	Low value of ratio
Presence or absence of Waist (W)	Presence	Absence
Relative length of the apical part (DR/PA)	PA short	PA large
	High value of ratio	Low value of ratio
Presence or absence of a blade (F)	Absence	Presence
Relative length of F (% of PA)	High value	Low value
Presence or absence of D	Absence	Presence
Number of D	Low number	High number
Number of rows of D	Few rows	More rows
Shape of D	small tubercles	Large, sharp
Basal angle of the serration (ABS)	< 45°	>45°
Presence or absence of a cusp (C)	Presence	Absence or transformation
Presence or absence of a basal spur	Presence	Absence
Width of the base (BA/DR)	Large base High value of ratio	Small base Low value of ratio
Shape of the base (BA)	Rectangular, large, obliquely elongate	Small, rounded

teeth, the cusp is changed early into a barb, as in *C. acutangulus* (Fig. 19) or in *C. praecellens* (Fig. 20). In the more typical vermivorous tooth, the cusp is present and usually small (i.e. in *C. flavusalbus* (Fig. 35), *C. ventricosus* (Fig. 36) and more prominent in other cases, as in *C. characteristicus* (Fig. 72), *C. borgesi* (Fig. 38) or *C. guinaicus* (Fig. 40).

Within a specialized line of vermivorous, (feeding on amphinomid worms) as *C. duffyi* (Fig. 73) and *C. imperialis* (Fig. 74) the prominent cusp is transformed into a barb. Also in the intermediate stage of piscivorous tooth the cusp is changed into a B3, i. e., *C. salzmanni* (Fig. 43) and in *C. jickeli* (Fig. 44) as it is well documented by the ontogenic change observed in *C. ermineus* (Fig. 67).

In the molluscivorous type of tooth, a cusp is always present and relatively prominent with respect to tooth width (Figs. 54-65).

Thus, the presence of a cusp is only evaluable in the primitive and in the vermivorous teeth, where its presence and its more prominent size is considered a derived state.

13. Presence or absence of a basal spur (SP)

Spur is defined as a distally oriented projection from the base of the radular tooth (PEILE, 1939).

A spur (SP) is absent in Drillidae, Clavatulidae and Crassispirinae. A spur is present in most species of higher turrids (Conidae *sensu* TAYLOR *et al.*, 1999), as Mangelinae (Figs. 3-5, 8-11), Clathurellinae and Onopotinae, though barely noticeable in some genera, as *Mitrolumna* (Figs. 6-7), or *Borsonella*, for example.

In *Conus* species, a basal spur (SP) appears in the most primitive type of teeth (Figs. 13-18); it is always present in the vermivorous (Figs. 34-40), and generalist type (Figs. 19-29), while it decreases in size in the large molluscivorous teeth (Figs.

54-65) and is definitively absent (lost) in the piscivorous teeth(Figs. 43-53).

The presence of SP, a shared state of the character of higher turrids, generalist and vermivorous *Conus* radular tooth, is here considered a primitive state of the character, therefore a plesiomorphy in *Conus.* Conversely, a very small sized or barely evident spur as well as the loss of a spur are derived states of the character.

14 Width of the base (BA/DR)
The width of the base has been defined as the ratio of base size on the radular tooth length (ROLÁN, 1992, ROLÁN & RAYBAUDI 1994). It coincides with the English translation of the terms proposed by KOHN *et al.* (1999).

The base is relatively larger in higher turrids teeth (Figs. 3-12), as well as in the primitive type of *Conus* teeth, (Figs. 16-18) in the generalist teeth (Figs. 19-27) and in the earlier developmental stages of the vermivorous teeth (Fig. 13-15, 34-35). BA decreases in the more typical type of vermivorous teeth (Figs. 36-40) and in the molluscivorous type of tooth (Figs. 54-65). Therefore the trend observed for width of the base is its decrease from the more primitive state of the character, shared by the great majority of *Conus* species, towards a derived state, shared by species of *Conus* with a molluscivorous and piscivorous type of teeth.

High values of this ratio are plesiomorphic, smaller ones are apomorphic.

15. Shape of the base (BA)
The base is relatively broad and often heavily reinforced in higher turrids as *Mangelia* (Figs. 3, 8-9), sometimes elongate (Figs. 4-5, 10-11), *Mitrolumna* (Figs. 6-7), *Genota* (Figs. 32-33). Furthermore, in primitive (Figs. 16-18) and older vermivorous (Fig. 13-15) it can be rounded; it is rectangular in some vermivorous lineages (Figs. 36-40). In piscivorous (Figs. 42-53) its shape is relatively small and rounded. In most generalist species (Figs. 19-27) is broad and its shape is obliquely elongated. In the molluscivorous tooth (Figs. 54-65) it is relatively small and rectangular. The shape and size of BA can define some lineage.

Thus, the broad rectangular and obliquely elongated base, a widespread state of the character in higher turrids and generalist, primitive and vermivorous types of *Conus* radular tooth, is a primitive state of the character in *Conus*. Conversely, a smaller and rounded base, a character shared by species of *Conus* with a molluscivorous and piscivorous type of tooth is an apomorphy.

DISCUSSION

Few molluscan groups suffered the difficulty to organize the available information from such a diverse sources as the Conidae. We face the problem raised from a large and taxonomically difficult group, with a rapidly increasingly number of species descriptions, for which an uncontestable stubborness of researchers led to maintain under a single genus as many as probably 750 living species, notwithstanding a stand-by taxa-park including 90 infra-sub-generic validly established names. The historical reasons of such a non-conventional approach (according to the ICZN standing rules) have been reviewed by DA MOTTA (1992) by ROCKEL *et al.* (1995) and by KOHN *et al.* (1999).

The very recent attempt of providing a first scheme of molecular phylogeny (ESPIRITU *ET AL.*, 2001), though evidently poorly resolved at the several nodes determinating clades, should stimulate researchers and it urgently calls for interdisciplinary debate and cooperation. Prey-capture mechanisms and trophic specialization are certainly essential determinants of the evolutionary success of this molluscan group. Both factors are clearly correlated with the sophisticated delivery of the complex and species-specific venom of *Conus*, by means of the highly transformed radular tooth. Thus, we confirm our belief (RAYBAUDI MASSILIA & ROLAN, 1995) that a phylogenetic scheme of the radular tooth will give an important contribution. Table V summarizes our conclusions on character state polarity for fifteen *Conus* radular tooth descriptors, based on the analysis of our data set. This set of characters and their polarity can be used in future for the elaboration of a cladistic analysis in order to compare the results with the molecular phylogenetic scheme (partial) proposed by Espiritu et al.2001.

Previous recent analysis (NISHI & KOHN, 1999, KOHN *et al.*, 1999) reviewed and defined a number of additional characters which may be useful for distinction at the species level and to discriminate subgroups within the five main types of radular tooth.

In the present work the known types of radular teeth have been shown and 15 characters and morphometric parameters employed in previous works to describe the different radula teeth were studied , determining the plesiomorphy or apomorphy of theit state.

As explained under Methods, we have maintained our original terms for the radular characters. Finally, we are more and more convinced that for a complete comparison, the use of camera lucida drawings is more useful than SEM photographs, because the latter may provide a good interpretation of the shape of the tooth, but does not allow to observe structure details which are extremely useful for comparative analysis.

Addendum
Information on the species whose radular teeth are presented with the indication of locality data of collected specimens.

Benthofascis sp. (from POWELL, 1966)
Conorbis coromandelicus E. A. Smith, 1894
Conus achatinus Gmelin, 1791, Thailand
Conus acuminatus Hwass, 1792, Dijbouti
Conus acutangulus Lamarck, 1810, Hawaii
Conus aphrodite Petuch, 1979, Philippines
Conus algoensis simplex Sowerby, 1857, South Africa
Conus amadis Gmelin, 1791, S. India
Conus ammiralis Linnaeus, 1758, Philippines
Conus ammiralis pseudocedonulli Blainville, 1818, Reunion Island

Conus arenatus Hwass, 1792, Comores Islands
Conus ateralbus Kiener, 1845, Cape Verde Archipelago
Conus australis Holten, 1802, Philippines
Conus babaensis Rolán & Röckel, 2001, Angola
Conus barbieri G. Raybaudi Massilia, 1995, Philippines
Conus barthelemyi Bernardi, 1861, Reunion
Conus belairensis Pin & Leung Tack, 1989, Senegal
Conus borgesi Trovão, 1979, Cape Verde Archipelago
Conus bozzettii Lauer, 1991, E. Somalia
Conus bulbus Reeve, 1843, Angola
Conus californicus Reeve, 1844, Gulf of California, USA
Conus carnalis Sowerby, 1879, Angola
Conus cedonulli Linné, 1767, Lesser Antilles
Conus chaldeus Röding, 1798, Pacific
Conus caracteristicus Dillwyn, 1817, Thailand
Conus coffeae Gmelin, 1791, Solomon Islands
Conus comatosa Pilsbry, 1904, Philippines
Conus cordigera Sowerby, 1866, Philippines.
Conus crocatus Lamarck, 1810, Solomon Is.
Conus curralensis Rolán, 1986, Cape Verde Archipelago
Conus cuvieri Crosse, 1858, Red Sea
Conus daucus Hwass, 1792, Brazil
Conus delanoyae Trovão, 1979, Cape Verde Archipelago
Conus delesserti Récluz, 1843, USA
Conus deyncerorum Petuch, 1995, Mexico
Conus diminutus Trovão & Rolán, 1986, Cape Verde Archipelago
Conus duffyi Petuch, 1992, Los Roques, Venezuela
Conus eburneus Hwass, 1792, Thailand
Conus echinophilus (Petuch, 1975), Senegal
Conus elegans Sowerby, 1895, Aden Gulf
Conus episcopatus Da Motta, 1982, Thailand
Conus ermineus Born, 1778, Senegal; Cape Verde Archipelago
Conus eugrammatus Bartsch & Rehder, 1943, Philippines
Conus eximius Reeve, 1849, Philippines
Conus fergusoni Sowerby, 1873, Gulf of California; Ecuador
Conus flavusalbus Rolán & Röckel, 2000, Angola
Conus franciscoi Rolán & Röckel, 2000, Angola
Conus friedae (Da Motta, 1991), Sri Lanka
Conus fuscolineatus Sowerby, 1905, Angola
Conus geographus Linnaeus, 1758, Solomon Islands
Conus gloriakiiensis Kuroda & Ito, 1961, Japan
Conus gondawanensis Röckel & Moolenbeek, 1995, New Caledonia
Conus guinaicus Hwass, 1792, Dakar, Senegal
Conus gubernator Hwass, 1792, Mozambique
Conus hieroglyphus Duclos, 1833, Nethelands Antilles
Conus imperialis Linnaeus, 1758, Reunion Island
Conus infrenatus Reeve, 1848, South Africa
Conus jaspideus Gmelin, 1791, Colombia; Bahamas
Conus jickeli Weinkauff, 1873, Djibouti
Conus julii Liénard, 1870, Reunion Island
Conus kimioi Habe, 1965, Philippines
Conus leehmani Da Motta & Röckel, 1979, Reunion Islands
Conus limpusi Röckel & Korn, 1990, Queensland
Conus lischkeanus Weinkauff, 1875, N. Somalia
Conus lividus Hwass, 1792, Hawaii
Conus lizarum Raybaudi & Da Motta, 1992, N. Somalia
Conus longurionis Kiener, 1845, Philippines
Conus lovellreveei G. Raybaudi Massilia, 1993, India
Conus lucidus Wood, 1828, Panama
Conus marmoreus Linnaeus, 1758, New Caledonia
Conus memiae Habe & Kosuge, 1970, Philippines
Conus mercator Linnaeus, 1758, Senegal
Conus miles Linnaeus, 1758, Reunion Island
Conus miliaris Hwass, 1792, Queensland, Australia.
Conus miruchae Röckel, Rolán & Monteiro, 1980, Cape Verde Archipelago
Conus moreleti Crosse, 1858, Hawaii
Conus monacus Linnaeus, 1758, Solomon Islands
Conus mucronatus Reeve, 1843, Philippines
Conus musicus Hwass, 1792, New Caledonia
Conus naranjus Trovão, 1975, Angola
Conus navarroi Rolán, 1986, Cape Verde Archipelago
Conus neoguttatus Da Motta, 1991, Angola
Conus nicobaricus Hwass, 1792, Philippines
Conus obscurus Sowerby, 1833, Reunion Island
Conus omaria Hwass, 1792, Philippines
Conus orbignyi Audouin, 1831, Philippines
Conus pagodus Kiener, 1845, Philippines
Conus papuensis Coomans & Moolenbeek, 1982, New Guinea
Conus patonganus da Motta, 1982, Thailand
Conus paulucciae Sowerby, 1876, Reunion Island
Conus pauperculus Sowerby I & Sowerby II, 1834, South Africa
Conus pealii Green, 1830, Florida
Conus pennaceus Born, 1778, Hawaii
Conus pineaui Pin, 1989, Senegal
Conus planorbis Born, 1778, Philippines
Conus praecellens A. Adams, 1854, Philippines
Conus profundorum (Kuroda, 1956), New Caledonia
Conus pulicarius Hwass, 1792, Hawaii
Conus queenslandis da Motta, 1984, Australia
Conus quercinus Lightfoot, 1786, Mozambique; Reunion Islands
Conus ranonganus da Motta, 1978. Andaman Sea; Solomon Islands
Conus reductaspiralis Walls, 1979, W. Australia
Conus ritae Petuch, 1995, Honduras
Conus rubropennatus Da Motta, 1982, Reunion Island
Conus rufimaculosus Macpherson, 1959, Queensland

Conus rutilus Menke, 1843, W. Australia
Conus salzmanni Raybaudi Massilia & Rolán, 1995, N. Somalia
Conus scalptus Reeve, 1843, Philippines
Conus segravei Gatliff, 1891, S. Australia
Conus sertacinctus Röckel, 1986, Solomon Islands
Conus shikamai Coomans & Moolenbeek, 1990, Philippines
Conus solomonensis Delsaerdt, 1992, Solomon Islands
Conus spectrum Linné, 1758, Queensland
Conus splendidulus Sowerby, 1833, Yemen
Conus stercusmuscarum Linnaeus, 1758, Solomon Islands
Conus stocki Coomans & Moolenbeek, 1990, Oman
Conus striatus Linnaeus, 1758, Philippines; Reunion Island
Conus sulcocastaneus Kosuge, 1981, Philippines
Conus tabidus Reeve, 1844, Cape Verde Archipelago
Conus teramachii Kuroda, 1956, Philippines
Conus terebra Born, 1778, Philippines
Conus terminus Lamarck, 1810, Reunion Island
Conus timorensis Hwass, 1792, Mauritius
Conus tribblei Walls, 1977, Philippines
Conus trochulus Reeve, 1844, Cape Verde Archipelago
Conus trovaoi Rolán & Röckel, 2000, Angola
Conus aff. vanhyningi Rehder, 1944, Aruba, Antilles
Conus vaubani Röckel & Moolenbeek, 1995, New Caledonia
Conus ventricosus Hwass, 1792, Portugal
Conus venulatus Hwass, 1792, Cape Verde Archipelago
Conus victoriae Reeve, 1843, W. Australia
Conus wallangra Garrard, 1961, W. Australia
Conus zapatosensis Röckel, 1987, Philippines
Conus zebroides Kiener, 1845, Angola
Crassispira callosa (Valenciennes, 1840), Ghana
Crassispira funebralis Fernandes, Rolán & Otero-Schmitt, 1995, Angola
Genota marchadi Pin, 1993, Senegal
Genota vafra Sykes, 1905, Angola
Mangelia albilonga Rolán & Otero-Schmitt, 1999, Angola
Mangelia congoensis Thiele, 1925, Angola
Mangelia digressa Rolán & Otero-Schmitt, 1999, Angola
Mangelia merlini Dautzenberg, 1910, Mauritania
Mangelia pontyi Dautzenberg, 1910, Mauritania
Mitrolumna monodi (Knudsen, 1956), Senegal
Mitrolumna saotomensis Rolán & Boyer, 2001, São Tomé Island

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